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(21) International Application Number: PCT/US95/04590 (22) International Filing Date: 13 April 1995 (13.04.95) (30) Priority Data: 229,276 14 April 1994 (14.04.94) US (60) Parent Application or Grant (63) Related by Continuation US 229,276 (CIP) Filed on 14 April 1994 (14.04.94) (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HUFF, Joel, R. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). LEE, Hee-Yoon [KR/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). NERENBERG, Jennie, B. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). THOMPSON, Wayne, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(74) Common Representative: MERCK & CO., INC.; Patent Dept., 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, US, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i>	
(54) Title: ALPHA1C ADRENERGIC RECEPTOR ANTAGONISTS			
(57) Abstract <p>This invention relates to certain novel compounds and derivatives thereof, their synthesis, and their use as selective alpha1C adrenergic receptor antagonists. One application of these compounds is in the treatment of benign prostatic hypertrophy. These compounds are selective in their ability to relax smooth muscle tissue enriched in the alpha1C receptor subtype without at the same time inducing orthostatic hypotension. One such tissue is found surrounding the urethral lining. Therefore, one utility of the instant compounds is to provide acute relief to males suffering from benign prostatic hyperplasia, by permitting less hindered urine flow. Another utility of the instant compounds is provided by combination with a human 5-alpha reductase inhibitory compound, such that both acute and chronic relief from the effects of benign prostatic hyperplasia are achieved.</p>			

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TITLE OF THE INVENTION

ALPHA1C ADRENERGIC RECEPTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

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1. Field of the Invention:

This application is a continuation-in-part of U.S. Serial No. 08/229,276, filed April 14, 1995, the contents of which are hereby incorporated by reference.

10

This invention relates to certain novel compounds and derivatives thereof, their synthesis, and their use as selective alpha-1c adrenoceptor antagonists. One application of these compounds is in the treatment of benign prostatic hypertrophy. These compounds are selective in their ability to relax smooth muscle tissue enriched in the alpha1C receptor subtype without at the same time inducing orthostatic hypotension. One such tissue is found surrounding the urethral lining. Therefore, one utility of the instant compounds is to provide acute relief to males suffering from benign prostatic hyperplasia, by permitting less hindered urine flow. Another utility of the instant compounds is provided by combination with a human 5-alpha reductase inhibitory compound, such that both acute and chronic relief from the effects of benign prostatic hyperplasia are achieved. Other advantages of the instant compounds are appreciated from the complete disclosure.

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ii. Background:

Human adrenergic receptors are integral membrane proteins which have been classified into two broad classes, the alpha and the beta adrenergic receptors. Both types mediate the action of the peripheral sympathetic nervous system upon binding of catecholamines, norepinephrine and epinephrine.

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Norepinephrine is produced by adrenergic nerve endings, while epinephrine is produced by the adrenal medulla. The binding affinity of adrenergic receptors for these compounds forms one basis of the classification: alpha receptors bind norepinephrine more strongly

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than epinephrine and much more strongly than the synthetic compound isoproterenol. The binding affinity of these hormones is reversed for the beta receptors. In many tissues, the functional responses, such as smooth muscle contraction, induced by alpha receptor activation are opposed to responses induced by beta receptor binding.

Subsequently, the functional distinction between alpha and beta receptors was further highlighted and refined by the pharmacological characterization of these receptors from various animal and tissue sources. As a result, alpha and beta adrenergic receptors were further subdivided into α_1 , α_2 , β_1 , and β_2 subtypes. Functional differences between α_1 and α_2 receptors have been recognized, and compounds which exhibit selective binding between these two subtypes have been developed. Thus, in WO 92/0073, the selective ability of the R(+) enantiomer of terazosin to selectively bind to adrenergic receptors of the alpha 1 subtype was reported. The α_1/α_2 selectivity of this compound was disclosed as being significant because agonist stimulation of the α_2 receptors was said to inhibit secretion of epinephrine and norepinephrine, while antagonism of the α_2 receptor was said to increase secretion of these hormones. Thus, the use of non-selective alpha-adrenergic blockers, such as phenoxybenzamine and phentolamine, is limited by their α_2 adrenergic receptor mediated induction of increased plasma catecholamine concentration and the attendant physiological sequelae (increased heart rate and smooth muscle contraction).

For a general background on the α -adrenergic receptors, the reader's attention is directed to Robert R. Ruffolo, Jr., α -Adrenoreceptors: Molecular Biology, Biochemistry and Pharmacology, (Progress in Basic and Clinical Pharmacology series, Karger, 1991), wherein the basis of α_1/α_2 subclassification, the molecular biology, signal transduction (G-protein interaction and location of the significant site for this and ligand binding activity away from the 3'-terminus of alpha adrenergic receptors), agonist structure-activity relationships, receptor functions, and therapeutic applications for compounds exhibiting α -adrenergic receptor affinity was explored.

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The cloning, sequencing and expression of alpha receptor subtypes from animal tissues has led to the subclassification of the α_1 receptors into α_1A , (Lomasney, et al., J. Biol. Chem., 266:6365-6369 (1991), rat α_1A ; Bruno et al., BBRC, 179:1485-1490 (1991), human α_1A), α_1B (Cotecchia, et al., PNAS, 85:7159-7163 (1988), hamster α_1B ; Libert, et al., Science, (1989), dog α_1B ; Ramarao, et al., J. Biol. Chem., 267:21936-21945 (1992), human α_1B), and most recently, in a study using bovine brain, a new α_1C subtype was proposed (Schwinn, et al., J. Biol. Chem., 265:8183-8189, 1990; Hirasawa et al., BBRC 195:902-909 (1993), described the cloning, functional expression and tissue distribution of a human α_1C adrenergic receptor; Hoehe et al., Human Mol. Genetics 1(5):349 (8/92) noted the existence of a two-allele PstI restriction fragment polymorphism in the α_1C adrenergic receptor gene; another study suggests that there may even be an alpha-1D receptor subtype, see Perez et al., Mol. Pharm., 40:876-883, 1992). Each α_1 receptor subtype exhibits its own pharmacologic and tissue specificities. Schwinn and coworkers noted that the cloned bovine α_1C receptor exhibited pharmacological properties proposed for the α_1A subtype. Nonetheless, based on its non-expression in tissues where the α_1A subtype is expressed, and its sensitivity to chloroethylclonidine, the receptor was given a new designation.

The differences in the α -adrenergic receptor subtypes have relevance in pathophysiologic conditions. Benign prostatic hypertrophy, BPH, is an illness typically affecting men over fifty years of age, increasing in severity with increasing age. The symptoms of the condition include, but are not limited to, increased difficulty in urination and sexual dysfunction. These symptoms are induced by enlargement, or hypertrophy, of the prostate gland. As the prostate increases in size, it impinges on free-flow of fluids through the male urethra. Concomitantly, the increased noradrenergic innervation of the enlarged prostate leads to an increased adrenergic tone of the bladder neck and urethra, further restricting the flow of urine through the urethra.

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The mechanism of prostatic hypertrophy is well understood. The male hormone, 5 α -dihydrotestosterone has been identified as the principal culprit. The continual production of 5 α -dihydrotestosterone by the male testes induces incremental growth of the prostate gland throughout the life of the male. Beyond the age of about fifty years, in many men, this enlarged gland begins to obstruct the urethra with the pathologic symptoms noted above.

The elucidation of the mechanism summarized above has resulted in the recent development of effective agents to control, and in many cases reverse, the pernicious advance of BPH. In the forefront of these agents is Merck & Co., Inc.'s product PROSCAR® (finasteride). The effect of this compound is to inhibit the enzyme testosterone 5-alpha reductase, which converts testosterone into 5 α -dihydrotestosterone, resulting in a reduced rate of prostatic enlargement, and often reduction in prostatic mass.

The development of such agents as PROSCAR® bodes well for the long-term control of BPH. However, as may be appreciated from the lengthy development of the syndrome, its reversal also is not immediate. In the interim, those males suffering with BPH continue to suffer, and may in fact lose hope that the agents are working sufficiently rapidly.

In response to this problem, one solution is to identify pharmaceutically active compounds which complement slower-acting therapeutics by providing acute relief. Agents which induce relaxation of the urethral smooth muscle, by binding to alpha-1 adrenergic receptors, thus reducing the increased adrenergic tone due to the disease, would be good candidates for this activity. Thus, one such agent is alfuzosin, which is reported in EP 0 204597 to induce urination in cases of prostatic hypertrophy. Likewise, in WO 92/0073, the selective ability of the R(+) enantiomer of terazosin to bind to adrenergic receptors of the α_1 subtype was reported. In addition, in WO 92/161213, hereby incorporated by reference, combinations of 5-alpha-reductase inhibitory compounds and alpha1-adrenergic receptor blockers (terazosin, doxazosin, prazosin, bunazosin, indoramin,

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alfuzosin) were disclosed. However, no information as to the α 1A, α 1B, or α 1C subtype specificity of these compounds was provided as these refinements were not yet available. Current therapy for BPH uses existing non-selective alpha-1 antagonists such as prazosin (Minipress, Pfizer) or Terazosin (Hytrin, Abbott). These non-selective antagonists suffer from side effects related to antagonism of the alpha-1a and alpha-1b receptors in the peripheral vasculature, eg., orthostatic hypotension and syncope.

Typically, identification of active compounds is through use of animal tissues known to be enriched in adrenergic receptors. Thus, rat tissues have been used to screen for potential adrenergic receptor antagonists. However, because of species variability, compounds which appear active in animal tissue may not be active or sufficiently selective in humans. This results in substantial wastage of time and effort, particularly where high volume compound screening programs are employed. There is also the danger that compounds, which might be highly effective in humans, would be missed because of their absence of appreciable affinity for the heterologous animal receptors. In this regard, it has been noted that even single amino acid changes between the sequence of biologically active proteins in one species may give rise to substantial pharmacological differences. Thus, Fong et al., (J. Biol. Chem., 267:25668-25671, 1992) showed that there are 22 divergent amino acid residues between the sequence of the human neurokinin-1 receptor and the homologous rat receptor. They further showed, in studies with mutant receptors, that substitution of only two amino acid residues was both necessary and sufficient to reproduce the rat receptor's antagonist binding affinity in the human receptor. Oksenberg et al., (Nature, 360:161-163, 1992) showed that a single amino-acid difference confers major pharmacological variation between the human and the rodent 5-hydroxytryptamine receptors. Likewise, Kuhse et al., (Neuron, 5:867-873, 1990) showed that a single amino-acid exchange alters the pharmacology of the neonatal rat glycine receptor subunit. This difficulty and unpredictability has resulted in a need for a

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compound screen which will identify compounds that will be active in humans.

These problems are solved by cloning the human adrenergic receptor of the α_1C subtype and the use of a screening assay
5 which enables identification of compounds which specifically interact with the human α_1C adrenergic receptor. Marshall *et al* (*Br. J. Pharm.*, 107:327 (1992)) speculated that compounds which specifically interact with the α_1C adrenergic receptor may be responsible for
10 contraction of the human prostate. As disclosed in the instant patent disclosure, a cloned human α_1C adrenergic receptor and a method for identifying compounds which bind the human α_1C receptor has now made possible the identification of specific human α_1C adrenergic
15 receptor antagonists. In the instant patent disclosure, we reveal novel compounds which we have discovered specifically bind the human α_1C receptor. These compounds are further tested for binding to other human alpha 1 receptor subtypes, as well as counterscreened against other types of receptors, thus defining the specificity of the compounds for the human α_1C adrenergic receptor.

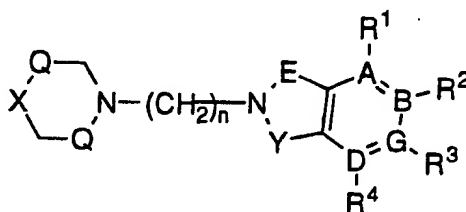
Compounds of his invention are used to reduce the acute
20 symptoms of BPH. Thus, compounds of this invention may be used alone or in conjunction with a more long-term anti-BPH therapeutics, such as testosterone 5-alpha reductase inhibitors, including PROSCAR® (finasteride). Aside from their utility as anti-BPH agents, these
25 compounds may be used to induce highly tissue-specific, localized α_1C adrenergic receptor blockade whenever this is desired. Effects of this blockade include reduction of intra-ocular pressure, control of cardiac arrhythmias, and possibly a host of alpha-1C receptor mediated central nervous system events.

30 SUMMARY OF THE INVENTION

This invention provides compounds for the treatment of urinary obstruction caused by benign prostatic hypertrophy (also known as benign prostatic hyperplasia or BPH). The compounds selectively antagonize the human alpha-1C adrenergic receptor at nanomolar and

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subnanomolar concentrations while exhibiting at least ten fold lower affinity for the $\alpha 1A$ and $\alpha 1B$ human adrenergic receptor and many other G-protein coupled receptors. This invention has the advantage over non-selective $\alpha 1$ adrenoceptor antagonists of reduced side effects related to peripheral adrenergic blockade. Such side effects include orthostatic hypotension, syncope, lethargy etc. These compounds have the structure:



and a pharmaceutically acceptable salt, prodrug, polymorph, or metabolite thereof wherein:

n is an integer from 3 to 5;

Y represents carbonyl, sulphonyl, $-\text{CO}-\text{CH}_2-$, or $-\text{CO}-\text{NR}^{12}-$;

R^{12} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted phenyl;

E is carbonyl or sulphonyl;

A, B, G, D are independently carbon or nitrogen;

R^1-R^4 are independently selected from the group consisting of hydrogen; halogen; nitro; amino; substituted or unsubstituted lower alkyl; perhalogenated lower alkyl; substituted or unsubstituted lower alkoxy; sulfonyl alkyl; and substituted or unsubstituted aryl or heteroaryl; with the proviso that if any of A, B, G, or D is a nitrogen, then the substituent R group is not present;

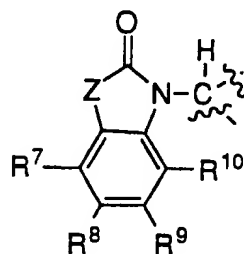
Q is, independently, $(-\text{CH}_2)_r$, $-\text{NH}-$, S, or O;

r is 0-3; and

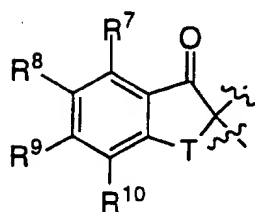
X is

a)

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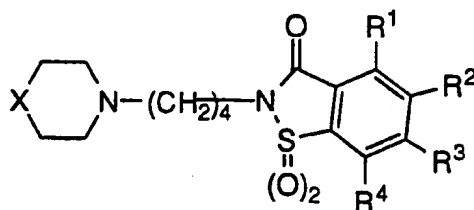
, or
b)



T is nitrogen, carbon, lower alkylene of one to three carbons or lower alkenylene of one to three carbons;

20 R⁷-R¹⁰ are independently selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, and lower alkoxy; and Z is O, S, CH₂, CH₂O, OCH₂, SCH₂, lower alkylene, lower alkenylene, NH, or NMe.

In one embodiment of the invention, the compound has the structure:



and a pharmaceutically acceptable salt, prodrug, polymorph or metabolite thereof, wherein all substituents are as defined above.

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5 These compounds may be used to advantage whenever specific blockade of the alpha1C adrenergic receptor is desirable, and are particularly useful in the treatment of benign prostatic hyperplasia (BPH) and for inhibiting contraction of prostate tissue, either alone or in combination with other active compounds. One preferred combination therapy includes the use of compounds described herein in conjunction with a compound effective to inhibit testosterone 5-alpha reductase.

10

BRIEF DESCRIPTION OF THE FIGURES

15 Fig. 1: Sequence of cDNA obtained by PCR of human heart mRNA, SEQ. ID:4:.

Fig. 2: Comparison of the open reading frame obtained from human heart, SEQ ID:5:, and the bovine alpha-1C adrenergic receptor sequence, SEQ. ID:6:.

20

Fig. 3: Sequence of cDNA obtained by screening a human hippocampus cDNA library using the heart mRNA derived sequence from figure 1, SEQ. ID:7:

25 Fig. 4: Sequence of 3' coding region of human alpha-1C gene, obtained by PCR amplification of a human genomic DNA library with oligonucleotides, SEQ. ID:10:.

30 Fig. 5: Sequence of the ligated portions of human alpha-1C DNA shown in figures 3 and 4, SEQ. ID:11:.

Fig. 6: The amino acid sequence of the human alpha-1C adrenergic receptor, SEQ ID:12:.

- 10 -

Fig. 7: The alignment of the nucleotide and amino acid sequence of the human alpha-1C adrenergic receptor, showing the 5'-untranslated region, SEQ. ID:11: and SEQ. ID:12:.

5 Fig. 8: Expression of the human alpha-1C adrenergic receptor in COS cells: Binding data using membranes from cells transfected with the expression vector alone and the expression vector containing the human alpha-1C adrenergic receptor coding sequences.

10 Fig. 9: Binding curves of compounds using membranes from COS cells transfected with the human alpha-1C adrenergic receptor containing expression vector.

15 Fig. 10: Nucleotide sequence of the human alpha1A receptor, SEQ. ID:13:

Fig. 11: Amino acid sequence of the human alpha1A adrenergic receptor, SEQ. ID:14:

20 Fig. 12: Partial sequence of the human alpha1B adrenergic receptor, SEQ. ID:17:

25 Fig. 13: Partial sequence of the human alpha1B adrenergic receptor, SEQ. ID:20:

Fig. 14: Partial sequence of the human alpha1B adrenergic receptor, SEQ. ID:23:

30 Fig. 15: Composite human/rat alpha1B adrenoreceptor, SEQ. ID:24:

Fig. 16: Amino acid sequence of the composite human/rat alpha1B adrenergic receptor, SEQ. ID:25:

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Fig. 17: Binding curves of compounds using membranes from COS cells transfected with the human $\alpha 1A$, $1B$, and $1C$ adrenergic receptor expression vectors.

5 Fig. 18: Sequence of truncated human $\alpha 1C$ adrenergic receptor, SEQ. ID:26:.

10 Fig. 19: Nucleotide sequence of the human $\alpha 1C$ adrenergic receptor having a Pst1 site, SEQ.ID:27:.

Fig. 20: Amino acid sequence of the human $\alpha 1C$ adrenergic receptor encoded by the Pst1 site encoding allele, SEQ.ID:28:.

15 Fig. 21: Alignment of the nucleotide and amino acid sequences of figures 19 and 20, SEQ.ID:27: and SEQ.ID:28:.

FIG. 22: Nucleotide sequence of the human $\alpha 1A$ adrenergic receptor, Seq.ID:29:.

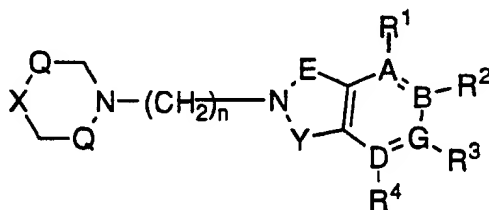
20 Fig. 23: Amino acid sequence of the human $\alpha 1A$ adrenergic receptor, SEQ.ID:30:.

25 Fig. 24: Alignment of the nucleotide and amino acid sequences of figures 22 and 23, SEQ.ID:29: and SEQ.ID:30:.

DETAILED DESCRIPTION OF THE INVENTION

Compounds of this invention have the structure:

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and a pharmaceutically acceptable salt, prodrug, polymorph, or metabolite thereof wherein:

n is an integer from 3 to 5;

5 Y represents carbonyl, sulphonyl, -CO-CH₂-, or -CO-NR¹²-;

R¹² is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted phenyl;

E is carbonyl or sulphonyl;

A, B, G, D are independently carbon or nitrogen;

10 R¹-R⁴ are independently selected from the group consisting of hydrogen; halogen; nitro; amino; substituted or unsubstituted lower alkyl; perhalogenated lower alkyl; substituted or unsubstituted lower alkoxy; sulfonyl alkyl; and substituted or unsubstituted aryl or heteroaryl; with the proviso that if any of A, B, G, or D is a nitrogen,

15 then the substituent R group is not present;

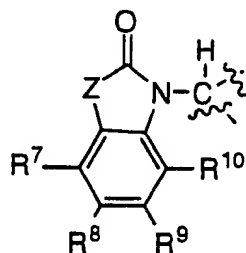
Q is, independently, (-CH₂-)_r, -NH-, S, or O;

r is 0-3; and

X is

a)

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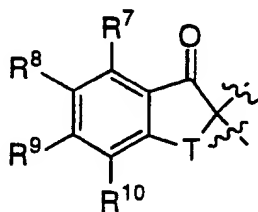


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, or

b)

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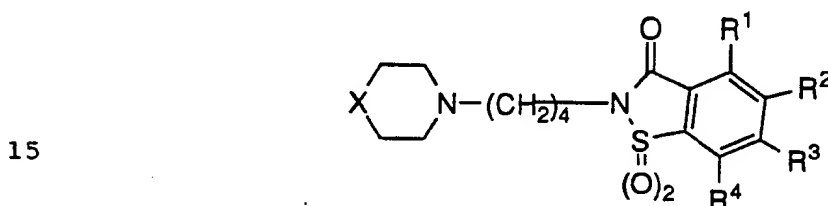


- 13 -

T is nitrogen, carbon, lower alkylene of one to three carbons or lower alkenylene of one to three carbons;

5 R⁷-R¹⁰ are independently selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, and lower alkoxy; and Z is O, S, CH₂, CH₂O, OCH₂, SCH₂, lower alkylene, lower alkenylene, NH, or NMe.

10 In one embodiment of the invention, the compound has the structure:



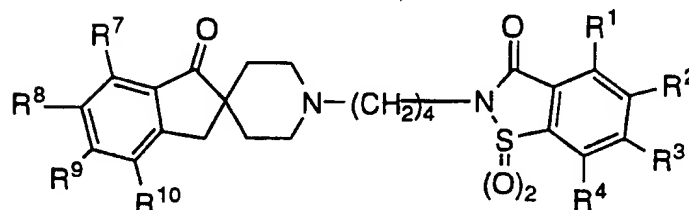
20 and a pharmaceutically acceptable salt, prodrug, polymorph or metabolite thereof, wherein all substituents are as defined above.

25 As used herein, the term lower alkyl, lower alkylene, or lower alkoxy means a substituted or unsubstituted, straight or branched chain of one to eight carbons. As used herein, a substituted group means that the group is halogenated, perhalogenated, particularly -CF₃, alkylated, alkoxylated, or is substituted with an aryl or heteroaryl. The term sulfonyl alkyl means a sulfonyl lower alkyl.

30 In a class of this embodiment of this invention, the compound is a piperidyl benzoxazinone substituted butyl saccharine or a spiroindanyl piperidine substituted butyl saccharine. In this embodiment of the invention, the compound is selected from:

- 14 -

5



and

10 wherein all variables are as previously defined. In specific embodiments of this invention, one of R^1 - R^4 is preferably an electron withdrawing group such as the nitro group, a halogen or a halogenated alkyl which substituents we have discovered contribute to improved bioavailability.

15 Representative compounds of this invention exhibit high selectivity for the human α_1C adrenergic receptor and are therefore useful for treating benign prostatic hyperplasia and for inhibiting contraction of prostate tissue. One implication of this selectivity for the human α_1C adrenergic receptor is that these compounds display
20 selectivity for lowering intraurethral pressure without substantially affecting diastolic blood pressure.

Representative compounds of this invention display submicromolar affinity for the human α_1C adrenergic receptor subtype while displaying at least ten-fold lower affinity for the human
25 α_1A and α_1B adrenergic receptor subtypes, and many other G-protein coupled human receptors. Particular representative compounds of this invention exhibit nanomolar and subnanomolar affinity for the human α_1C adrenergic receptor subtype while displaying at least 30 fold lower affinity for the human α_1A and α_1B adrenergic
30 receptor subtypes, and many other G-protein coupled human receptors. Representative compounds of this invention exhibit K_i 's for human α_1C adrenergic receptors over a 500 fold lower than for the human α_1A or α_1B adrenergic receptors, while exhibiting greater than 300 fold selectivity for the human α_1C adrenergic receptor over all other human G-protein coupled receptors tested (including serotonin,

- 15 -

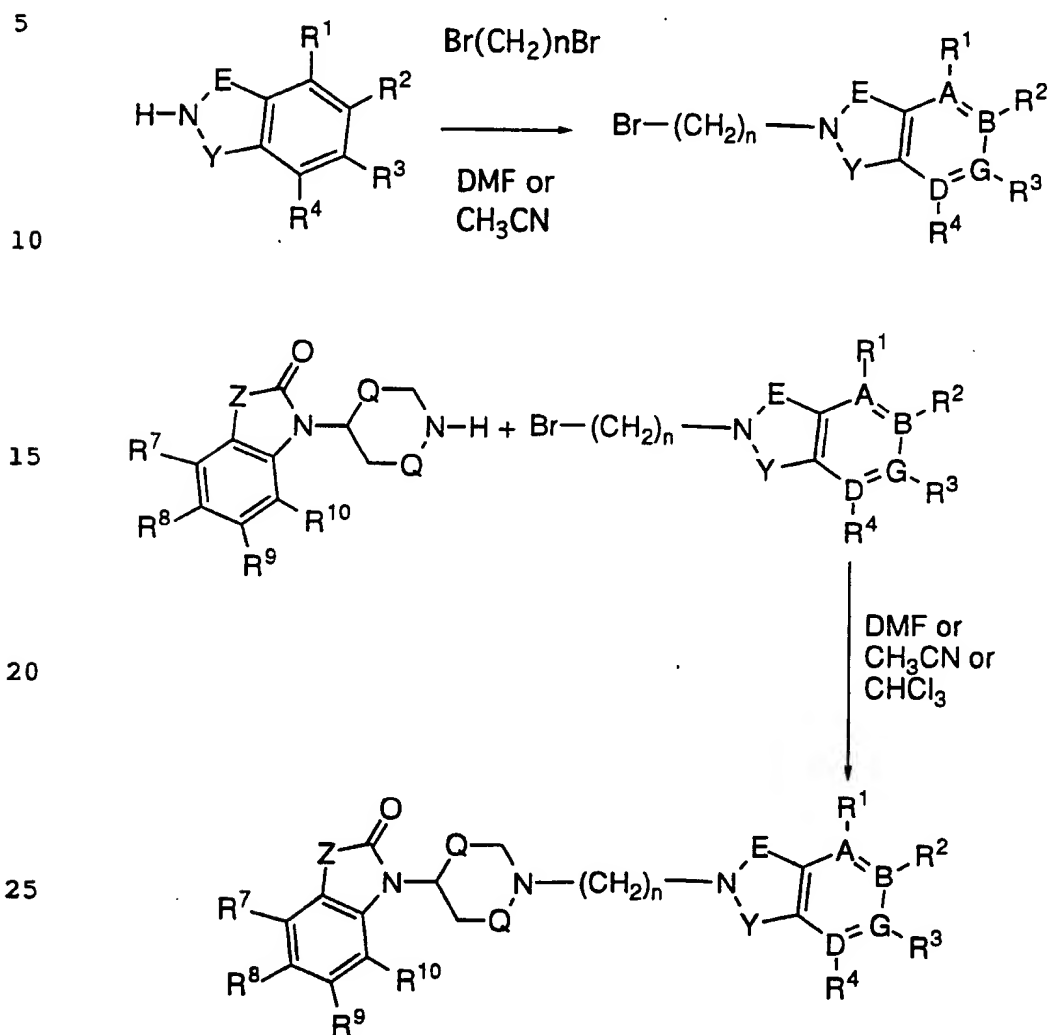
dopamine, alpha 2 adrenergic, beta adrenergic or muscarinic receptors). Furthermore, representative compounds of this invention exhibit good bioavailability in known animal models (approximately 30% in dogs and 16% in rats). These indicia are expected to provide good indications of bioavailability in humans.

Compounds of this invention are prepared by a two step alkylation process beginning with a saccharine or substituted saccharine (referred to as "the saccharine moiety") prepared according to methods known in the art (see for example US Patents 4,818,756; 4,937,343; 4,988,809 and 5,187,276, hereby incorporated by reference for this purpose). Preparation of spiropiperidines used herein in the preparation of compounds of this invention is described, for example, in published European Patent Application 90313262.9 (publication number 0 431 943 A2, 6/12/91, herein incorporated by reference for this purpose). The saccharine moiety is alkylated with a reagent such as 1,4-dibromobutane, or a similar reagent, to form the butyl-saccharine moiety (see Scheme 1 below). The butyl saccharine is then alkylated with a piperidinyl benzoxazinone (prepared as shown in Scheme 2 below) or with a spiroindanyl piperidine (prepared as shown in Scheme 3 below) to form the active compounds of this invention (see Scheme 1 below). These steps are further defined with reference to the following schemes, and the synthetic examples appended hereto. It should be understood that the specific solvents, catalysts and reactants could be substituted by analogous reagents by those skilled in the art. All substituents are as defined above:

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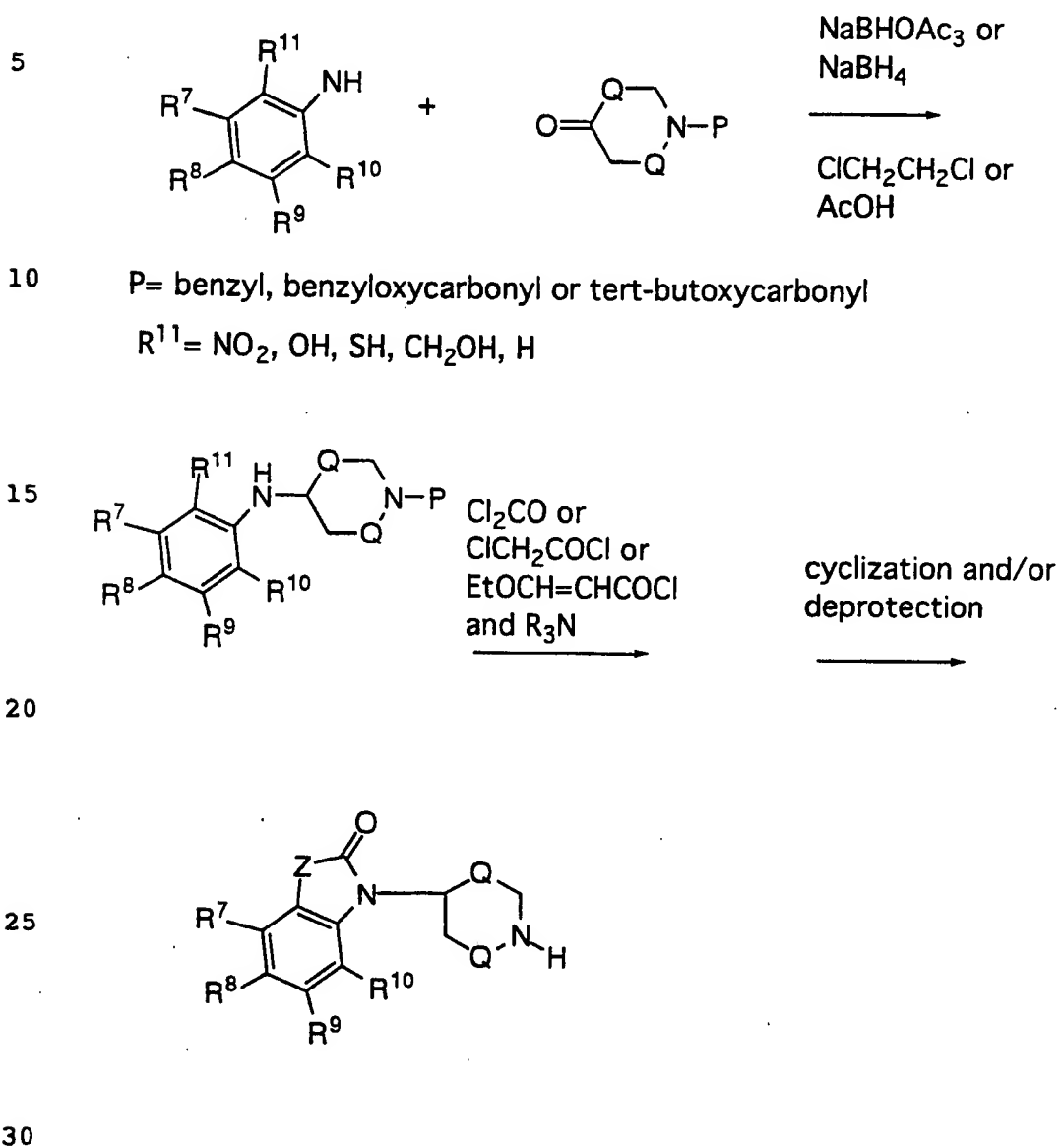
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Scheme 1



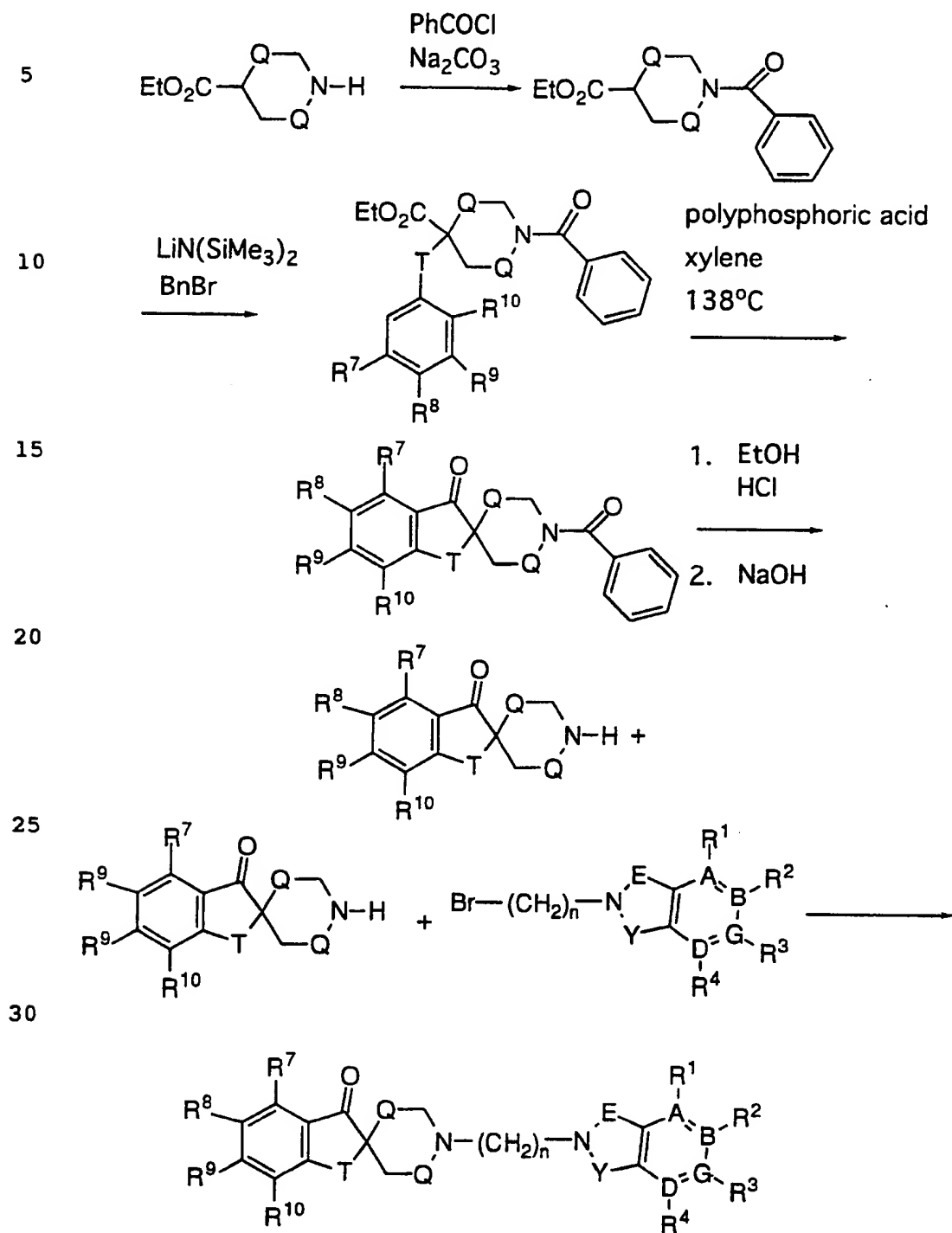
- 17 -

Scheme 2



- 18 -

Scheme 3



- 19 -

Abbreviations: DMF is dimethylformamide; EtOH is ethanol.

These compounds are administered in dosages effective to antagonize the $\alpha 1C$ receptor where such treatment is needed, as in BPH and for inhibiting contraction of prostate tissue. For use in
5 medicine, the salts of the compounds of this invention will be pharmaceutically acceptable salts. Other salts may, however, be useful in the preparation of the compounds according to the invention or of their pharmaceutically acceptable salts. Suitable pharmaceutically acceptable salts of the compounds of this invention include acid addition
10 salts which may, for example, be formed by mixing a solution of the compound according to the invention with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulphuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, oxalic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid.
15 Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts, e.g. sodium or potassium salts; alkaline earth metal salts, e.g. calcium or magnesium salts; and salts formed with suitable organic ligands, e.g. quaternary ammonium salts.

20 The present invention includes within its scope prodrugs of the compounds of this invention. In general, such prodrugs will be functional derivatives of the compounds of this invention which are readily convertible in vivo into the required compound. Conventional procedures for the selection and preparation of suitable prodrug
25 derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985. Metabolites of these compounds include active species produced upon introduction of compounds of this invention into the biological milieu.

30 Where the compounds according to the invention have at least one asymmetric centre, they may accordingly exist as enantiomers. Where the compounds according to the invention possess two or more asymmetric centres, they may additionally exist as diastereoisomers. It is to be understood that all such isomers and mixtures thereof are encompassed within the scope of the present invention. Furthermore,

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some of the crystalline forms for compounds of the present invention may exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the compounds of the present invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of this invention.

Specific compounds within the scope of the present invention include but are not limited to:

- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(3,4-dihydro-2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3,1,4-benzoxazinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;
- 6-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 5-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-7-nitro-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;
- 6-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 5-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-7-nitro-1,2-benzisothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(5-chloro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 5 1,1-Dioxido-2-(4-(4-(3a-(R)-8a-(S)-2-oxo-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxonaphth[2,3-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 10 1,1-Dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-5-phenyl-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 15 1,1-Dioxido-2-(4-(4-(6-methoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(6-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(5-ethylsulfonyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 20 1,1-Dioxido-2-(4-(4-(2-oxo-3-oxazolo[4,5-b]pyridyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(7-carbethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 25 1,1-Dioxido-2-(4-(4-(5-*tert*-butyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(5,7-dimethyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one;
- 30 1,1-Dioxido-2-(4-(4'-(3,4-dihydro-1-oxonaphthalene)-2(1H)-spiropiperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one;
- 4-(3,4-Dihydro-6-methyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide;

- 4-(Spiro(piperidine-4,6'-[6H]thieno[2,3-b]thiopyran-4'(5'H)-one-1'-yl)-butylphthalimide;
- 4-(Spiro[benzothiazol-2(3H),4'-piperidin-1'-yl]-butylphthalimide;
- 5 4-(3,4-Dihydro-6-methoxy-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide;
- 4-(3,4-Dihydro-6-methanesulfonylamidyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide;
- 1,1-Dioxido-2-(4-(spiro[benzothiazol-2(3H),4'-piperidin-1'-yl]-butyl)-1,2-benzisothiazol-3(2H)-one;
- 10 4-(6-Trifluoromethyl-spiro[benzothiazol-2(3H),4'-piperidin-1'-yl]-butylphthalimide;
- 1,1-Dioxido-2-(4-(spiro[benzofuran-2(3H),4'-piperidin]-1'-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 4-(Spiro[benzofuran-2(3H),4'-piperidin]-1'-yl)-butylphthalimide;
- 15 4-(Spiro[2H-1,3-benzoxazine-2,4'-piperidin]-1'-yl)-butylphthalimide;
- 3,3-Dioxido-1,2-dehydro-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-naphth[1,2-d]isothiazol-1-one;
- 1,1-Dioxido-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one;
- 20 1,1-Dioxido-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-7-methoxy-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-methoxy-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-6-methoxy-1,2-benzisothiazol-3(2H)-one;
- 25 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-methyl-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
- 30 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-6-nitro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one;
5 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one ;
1,1-Dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;
2-(4-(Spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-pyrrolo[3,4b]pyridin-5,7(1H)-dione;
10 1,1-Dioxido-2,3-dihydro-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-naphth[1,8-de]isothiazin-3-one; and
1,1-Dioxido-2-(4-(spiro[3-oxo-phthalan-1,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one;
15 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-3-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
20 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-4-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methoxy-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
25 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-chloro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-fluoro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
30 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-6-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-1H-3,4-dihydroquinazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 2-(4-Hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-d]benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-ethoxy-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-ethoxy-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-ethyl-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-ethyl-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-(2-propyl)-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-(2-propyl)-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-6-nitro-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-methyl-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-bromo-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-trifluoromethyl-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-trifluoromethoxy-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-1-naphth[1,2-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(4-(5,6,7,8-tetrahydro-2-oxo-3-naphth[2,3-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5 1,1-Dioxido-2-(4-(4-(5-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(6-fluoro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
10 1,1-Dioxido-2-(4-(4-(4-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(4-methoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Chloro-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
15 5-Methylthio-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Ethoxy-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Chloro-1,1-dioxido-2-(4-(4-(6-fluoro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
20 4-Methyl-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
4-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
25 4-Ethoxy-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
4-(2-Propyloxy)-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Methoxy-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
30 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-6-methylsulfonyl-1,2-benzisothiazol-3(2H)-one;
5-Methoxy-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

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1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-methylsulfonyl-1,2-benzisothiazol-3(2H)-one;

5-Methylthio-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

5 5-Methylsulfonyl-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one; or
1,1-Dioxido-2-(4-(4-(2-oxo-1-oxazolo[5,4-b]pyridyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

and pharmaceutically acceptable salts, metabolites and prodrugs thereof.

10 Preferred compounds of the present invention include:

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-3-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

15 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-chloro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

20 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-fluoro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;

25 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-6-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;

and

1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;

and pharmaceutically acceptable salts, metabolites and prodrugs thereof.

30 The invention also provides pharmaceutical compositions comprising one or more compounds of this invention in association with a pharmaceutically acceptable carrier. Preferably these compositions are in unit dosage forms such as tablets, pills, capsules, powders, granules, sterile parenteral solutions or suspensions, metered aerosol or liquid sprays, drops, ampoules, auto-injector devices or suppositories;

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for oral, parenteral, intranasal, sublingual or rectal administration, or for administration by inhalation or insufflation. Alternatively, the compositions may be presented in a form suitable for once-weekly or once-monthly administration; for example, an insoluble salt of the active compound, such as the decanoate salt, may be adapted to provide a depot preparation for intramuscular injection. For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical carrier, e.g. conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g. water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavoured syrups, aqueous

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or oil suspensions, and flavoured emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil or peanut oil, as well as elixirs and similar pharmaceutical vehicles. Suitable dispersing or suspending agents for aqueous suspensions include synthetic and natural gums such as tragacanth, acacia, alginate, dextran, sodium carboxymethylcellulose, methylcellulose, polyvinyl-pyrrolidone or gelatin.

In the treatment of BPH, a suitable dosage level is about 0.01 to 250 mg/kg per day, preferably about 0.05 to 100 mg/kg per day, and especially about 0.05 to 5 mg/kg per day. The compounds may be administered on a regimen of 1 to 4 times per day.

Where the above-described processes for the preparation of the compounds according to the invention give rise to mixtures of stereoisomers, these isomers may be separated by conventional techniques such as preparative chromatography. The compounds may be prepared in racemic form, or individual enantiomers may be prepared either by enantiospecific synthesis or by resolution. The compounds may, for example, be resolved into their component enantiomers by standard techniques, such as the formation of diastereomeric pairs by salt formation with an optically active acid, such as (-)-di-p-toluoyl-d-tartaric acid and/or (+)-di-p-toluoyl-l-tartaric acid followed by fractional crystallization and regeneration of the free base. The compounds may also be resolved by formation of diastereomeric esters or amides, followed by chromatographic separation and removal of the chiral auxiliary.

During any of the above synthetic sequences it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups, such as those described in Protective Groups in Organic Chemistry, ed. J.F.W. McOmie, Plenum Press, 1973; and T.W. Greene & P.G.M. Wuts, Protective Groups in Organic Synthesis, John Wiley & Sons, 1991. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

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The specificity of binding of compounds showing affinity for the $\alpha 1C$ receptor is shown by comparing affinity to membranes obtained from COS cells transfected with the cloned $\alpha 1C$ receptor and membranes from tissues known to express other types of alpha or beta adrenergic receptors. In addition, the cloned human $\alpha 1A$ and a hybrid human/rat $\alpha 1B$ (with only the cytoplasmic, carboxy terminal region being rat sequence) could be used for this purpose, along with the human $\alpha 1C$ receptor expressed in COS cells. Expression of the cloned human $\alpha 1A$, $\alpha 1B$, and $\alpha 1C$ receptors and comparison of their binding properties with known selective antagonists provides a rational way for selection of compounds and discovery of new compounds with predictable pharmacological activities. Antagonism by these compounds of the human $\alpha 1C$ adrenergic receptor subtype may be functionally demonstrated in anesthetized animals. These compounds may be used to increase urine flow without exhibiting orthostatic hypotensive effects.

The human alpha adrenergic receptor of the 1-C subtype was identified, cloned and expressed. A partial coding region for this receptor was generated by reverse transcriptase-polymerase chain reaction technology, RT-PCR. Accordingly, degenerate oligonucleotides encoding amino acids conserved in the fifth and sixth transmembrane domains of all three $\alpha 1$ receptor subtypes (A, B, C) were used to prime RT-PCR reactions using human heart mRNA as template. The predicted sized products were cloned and sequenced. Translation of the amplified cDNA yielded an open reading frame encoding a protein 95% homologous to the bovine $\alpha 1C$ receptor (Fig.2, SEQ. ID:5: and SEQ. ID:6:). This partial sequence was used to obtain a larger cDNA clone from a human hippocampus library (Fig. 3, SEQ. ID:6:). The remaining coding region was obtained by PCR amplification of human genomic DNA using primers based on the cDNA sequence and the last six amino acids of bovine $\alpha 1C$ receptor (Fig. 4, SEQ.ID:10:). The complete receptor was then assembled using the partial sequences shown in Fig. 3, SEQ.ID:6: and Fig. 4, SEQ. ID:10:, to generate the sequence shown in Fig. 5, SEQ. ID:11:. The translation of this sequence is shown in Fig. 6, SEQ. ID:12:, and the

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alignment of the nucleotide and amino acid sequences, and the 5'-untranslated sequences, is shown in Fig. 7, SEQ. ID:11: and SEQ. ID:12:.

5 The 3'-terminal six amino acids of the human $\alpha 1C$
adrenergic receptor were confirmed by screening a human genomic
library with the radiolabeled 3'-terminal 512 nucleotides of the SEQ.
ID:10: clone previously obtained. A complete human exon 2 was
generated in this manner and sequenced. The nucleotide sequence of
10 this gene is provided in figure 19, SEQ. ID:27: and the amino acid
sequence is provided in figure 20, SEQ. ID:28:. We discovered that this
clone was identical to the original 3'-terminal portion of the gene,
except that:

- 1) There are five silent nucleotide changes between the new clone and
15 the previously obtained clone (the last five codons, including the stop
codon, each have a silent change in the third nucleotide); and
- 2) At nucleotide position 1636 (amino acid 347), there is a cytosine to
thymine base change resulting in the formation of a PstI site at that
location and a concomitant single amino acid change of Arg to Cys.
20 Thus, we have confirmed and localized the site of the two-allele PstI
restriction fragment polymorphism (RFLP) noted by Hoehe et al.,
[Human Mol. Genetics, 1(5):349 (8/92)]. Through pharmacological
studies using clones of both alleles, we have confirmed that the Arg to
Cys change appear to be pharmacologically indistinguishable (see Table
25 II, Example 11, below).

25 The cloned human $\alpha 1C$ receptor, when expressed in
mammalian cell lines (see Fig. 8), is used to discover ligands that bind
to the receptor and alter its function. In addition, the cloned $\alpha 1C$
receptor enables quantitation of mRNA levels in human tissues,
including the aorta and prostate, by RNase protection assays. For these
30 purposes, a complete coding sequence of the receptor is provided.
However, as long as the ligand binding and signal transduction segments
of the receptor (G-protein interaction) are intact, truncation at the 3'
end of the sequence does not affect the functioning of the receptor.
Thus, in addition to the sequence provided in SEQ. ID:11:, a sequence,

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truncated at the 3' end, SEQ. ID:26: is disclosed, which consists entirely of human $\alpha 1C$ sequence.

Once the human receptor is cloned and expressed in a cell such as COS cells or CHO cells, the receptor is free of other human proteins. The membranes from cells expressing different human α adrenergic receptor subtypes are then isolated according to methods well known in the art for membrane associated receptor binding assays. For example, the method of Schwinn, et al., (J. Biol. Chem., 265:8183-8189, 1990) may be used. A compound of interest is used to compete with the binding of a known, quantifiable α receptor ligand. Thus, radiolabeled prazosin, niguldipine, 5-methyl urapidil, terazosin, doxazosin, phenoxybenzamine, WB4101, benoxathian, HEAT (2-[β -(4-hydroxy-3-iodophenyl)ethylaminomethyl]tetralone, or phentolamine may be used for this purpose (see, for example, Robert R. Ruffolo, Jr., α -Adrenoreceptors: Molecular Biology, Biochemistry and Pharmacology, (Progress in Basic and Clinical Pharmacology series, Karger, 1991), page 29). Because of the ease of 125 Iodine detection, ^{125}I -HEAT may be preferred for this purpose. By increasing the amount of unlabeled, test compound, the labeled compound is competed off the receptor. From these experiments, IC_{50} values for each test compound and receptor subtype is determined.

A new sequence for the human $\alpha 1A$ adrenergic receptor which is more homologous to the rat $\alpha 1A$ adrenergic receptor sequence is also provided herein (see Example 12 and figures 22, 23, and 24, SEQ. ID:29: and SEQ. ID:30:). While no difference in ligand binding has thus far been observed based on the different amino terminal amino acid sequences between these two receptors, such differences cannot be ruled out except by screening compounds against both clones. Since a new human $\alpha 1A$ adrenergic receptor sequence is provided herein, compounds identified using the earlier reported human $\alpha 1A$ adrenergic receptor sequence can now be confirmed against this clone.

Compounds of this invention exhibiting selective human $\alpha 1C$ adrenergic receptor antagonism may further be defined by counterscreening. This is accomplished according to methods known in

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the art using other receptors responsible for mediating diverse biological functions. Compounds which are both selective amongst the various human $\alpha 1$ adrenergic receptor subtypes and which have low affinity for other receptors, such as the $\alpha 2$ adrenergic receptors, the β -adrenergic receptors, the muscarinic receptors, the serotonin receptors, and others are particularly preferred. The absence of these non-specific activities may be confirmed by using cloned and expressed receptors in an analogous fashion to the method disclosed herein for identifying compounds which have high affinity for the various human $\alpha 1$ adrenergic receptors. Furthermore, functional biological tests are used to confirm the effects of identified compounds as $\alpha 1$ adrenergic receptor antagonists.

Compounds of this patent disclosure may be used alone at appropriate dosages defined by routine testing in order to obtain optimal antagonism of the human $\alpha 1$ adrenergic receptor while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents which alleviate the effects of BPH is desirable. Thus, in one embodiment, this includes administration of compounds of this invention and a human testosterone 5- α reductase inhibitor. Included in this embodiment are inhibitors of 5- α reductase isoenzyme 2. Many such compounds are now well known in the art and include such compounds as PROSCAR®, (also known as finasteride, a 4-Aza-steroid; see US Patents 4,377,584 and 4,760,071, for example, hereby incorporated by reference). In addition to PROSCAR®, which is principally active in prostatic tissue due to its selectivity for human 5- α reductase isozyme 2, combinations of compounds which are specifically active in inhibiting testosterone 5- α reductase isozyme 1 (found particularly in skin) and compounds which act as dual inhibitors of both isozymes 1 and 2, are useful in combination with compounds of this invention. Compounds that are active as 5 α -reductase inhibitors have been described in WO93/23420, EP 0572166; WO 93/23050; WO93/23038, ; WO93/23048; WO93/23041; WO93/23040; WO93/23039; WO93/23376;

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WO93/23419, EP 0572165; WO93/23051, each of which is hereby incorporated by reference.

5 The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds of this invention as the active ingredient for use in the specific antagonism of human
10 $\alpha 1C$ adrenergic receptors can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for systemic administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by
15 injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be
20 employed as an $\alpha 1C$ antagonistic agent.

The daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult human/per day. For oral administration, the compositions are preferably provided in the form of scored or unscored tablets containing 0.01, 0.05, 0.1, 0.5,
25 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0002 mg./kg to about 50 mg./kg. of body weight per day. The range is more particularly from about 0.001 mg./kg to 7 mg./kg. of body weight per day. With
30 reference to terazosin, it is predictable that the potent and more selective compounds of this invention are effective at doses equal to or between ten and a hundred fold lower than dosages utilized for that compound (see, for example, US Patent 5,212,176). The dosages of the $\alpha 1C$ adrenergic receptor and testosterone 5- α

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reductase inhibitors are adjusted when combined to achieve desired effects. As those skilled in the art will appreciate, less 5-alpha reductase inhibitor may be required when the acute symptoms of BPH are alleviated by treatment with the alpha1C adrenergic
5 receptor inhibitor of this invention. On the other hand, dosages of these various agents may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if either agent were used alone.

10 The term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes
15 alleviation of the symptoms of the disease being treated. The term inhibitorily effective amount or antagonistically effective amount as used herein means that amount of an active compound which, when contacted with a particular enzyme or receptor, interrupts the usual catalytic activity or signal transduction of that enzyme or receptor.

20 The term "subject," as used herein refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times
25 daily. Furthermore, compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the
30 dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For the treatment of benign prostatic hyperplasia, prostatitis and the prevention and/or treatment of prostatic cancer, compounds of this invention exhibiting alpha1C adrenergic receptor

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blockade can be combined with a therapeutically effective amount of a 5α -reductase 2 inhibitor, such as finasteride, in addition to a 5α -reductase 1 inhibitor, such as 4,7 β -dimethyl-4-aza- 5α -cholestan-3-one, in a single oral, systemic, or parenteral pharmaceutical dosage formulation. Alternatively, a combined therapy can be employed wherein the $\alpha 1C$ adrenergic receptor antagonist and the 5α -reductase 1 or 2 inhibitor are administered in separate oral, systemic, or parenteral dosage formulations. See, e.g., U.S. Patent No.'s 4,377,584 and 4,760,071 which describe dosages and formulations for 5α -reductase inhibitors.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

In the methods of the present invention, the compounds herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups

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and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with
5 an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable
10 binders include, without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, without limitation,
15 sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

The liquid forms in suitably flavored suspending or dispersing agents such as the synthetic and natural gums, for example,
20 tragacanth, acacia, methyl-cellulose and the like. Other dispersing agents which may be employed include glycerin and the like. For parenteral administration, sterile suspensions and solutions are desired. Isotonic preparations which generally contain suitable preservatives are employed when intravenous administration is desired.

25 The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

30 Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-

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pyrrolidone, pyran copolymer, polyhydroxypropylmethacryl-
amidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyl-
eneoxidepolylysine substituted with palmitoyl residues.

5 Furthermore, the compounds of the present invention may be
coupled to a class of biodegradable polymers useful in achieving
controlled release of a drug, for example, polylactic acid,
polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters,
polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked
10 or amphipathic block copolymers of hydrogels.

Compounds of this invention may be administered in
any of the foregoing compositions and according to dosage regimens
established in the art whenever specific blockade of the human
alpha1C adrenergic receptor is required.

15 The following examples are provided to further define the
invention without, however, limiting the invention to the particulars of
these examples.

20 EXAMPLE 1

PCR amplification, cloning and sequencing of ph α 1X:

25 Based on the amino acid homologies of human α 1A, rat α 1B and bovine
 α 1C receptors, degenerate oligonucleotides were designed to amplify
cDNAs encoding all three receptor subtypes. These oligonucleotides
are:

30 WL' (SEQ. ID:1) TTTTCTAGAT TRTTNARRTA NCCNAGCC 28

MYC (SEQ. ID:2) TTTACTAGTA TCSTNGTNAT GTAYTG 16

WC' (SEQ. ID:3) TTTTCTAGAG AARAANGGNA RCCARC 26

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Oligonucleotides MYC and WL' were used as primers in a reverse transcription PCR amplification of human heart mRNA (Clontech) using the RNA PCR kit from Perkin Elmer Cetus. Briefly, 0.5 ug of mRNA was reverse transcribed in a volume of 20 ul using either random oligonucleotide primers (reaction 1) or oligo dT primer (reaction 2). Reactions 1 and 2 were pooled and served as template for PCR amplification as follows:

PCR Reactions:

10

Primary reaction (50 ul)

5 ul 10X buffer from Perkin Elmer Cetus GeneAmp Kit
8 ul 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
3ul first strand cDNA
15 1 ul 25 pMoles oligo MYC
1 ul 25 pMoles oligo WL'
0.25 ul 1.25 units Amplitaq DNA polymerase
31.75 ul water

20

Reaction conditions; 40 cycles of 94°C 1'; 45°C 2'; 72°C 2'

Secondary reaction (100 ul)

9.5 ul 10X buffer from Perkin Elmer Cetus GeneAmp Kit
16ul 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
25 5ul first strand cDNA
2 ul 50 pMoles oligo MYC
2 ul 50 pMoles oligo WC'
0.5 ul 2.5 units Amplitaq DNA polymerase
65 ul water

30

Reaction conditions; 40 cycles of 94°C 1'; 45°C 2'; 72°C 2'

Prep scale tertiary reaction 3 X 200 ul:

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19.5 ul 10X buffer
32 ul 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
5 ul secondary PCR reaction
4 ul 100 pMoles oligo MYC
5 4 ul 100 pMoles oligo WC'
1 ul 5 units Amplitaq DNA polymerase
134.5 ul water

10 Reaction conditions; 30 cycles of 94°C 1'; 50°C 2'; 72°C 2'

The PCR product was purified by Qiagen spin columns and digested with restriction endonucleases SpeI and XbaI. The fragment was then ligated into SpeI/XbaI cut pGEM9Zf(-). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from
15 white transformants and sequenced by the dideoxy chain termination method. The base sequence obtained is shown in Fig. 1, SEQ. ID:4:.

EXAMPLE 2

20 Isolation of partial alpha1C cDNA Clone:

A cDNA library prepared from mRNA isolated from human hippocampus (Stratagene) was screened by plaque hybridization using
25 phα1X as a probe. Hybridization conditions were as follows:

5XSSC (1XSSC is 0.15M sodium chloride, 0.015M sodium
citrate,
50% Formamide
30 5X Denhardt's Solution (1% Ficoll, 1%
polyvinylpyrrolidone, 1% bovine serum albumin)
0.15 mg/ml salmon sperm DNA
hybridize overnight at 42° C.

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Filters were washed 3 times in 2XSSC, 0.1% SDS at room temperature for 5', then 1 time in 1XSSC, 0.1% SDS at 50C for 30'. Positive clones were identified by autoradiography. Phagemid DNA was rescued from the positive plaques and sequenced by the dideoxy chain termination method. The base sequence obtained is shown in Fig. 3, SEQ. ID:7:.

EXAMPLE 3

10 PCR amplification, cloning and sequencing of 3'CG of alpha1C:

The 3' end of the coding region of human alpha1C adrenergic receptor was amplified from human genomic DNA using two oligonucleotides:

15

S3C (SEQ ID:8:)

5' TTTGAATTCT GATTTC AAGC CCTCTG 3'

20

and

3'C (SEQ ID:9:)

25

5' TTTGAATTCT TANACYTCYT CNCCRTTYTC 3'

as follows:

30 10 ul 10X buffer from Perkin Elmer Cetus GeneAmp Kit
16 ul 1.25 mM each stock of dATP,dCTP,dGTP, and dTTP
6 ul 1 ug human genomic DNA (Promega)
2 ul 50 pMoles oligo S3C
2 ul 50 pMoles oligo 3'C
0.5 ul 2.5 units Amplitaq DNA polymersase
63.5 ul water

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Reaction conditions; 40 cycles of 94°C 1'; 50°C 2';
72°C 2'

5 The PCR product was purified by Qiagen spin columns and
digested with restriction endonuclease EcoRI. The fragment was then
ligated into EcoRI cut pGEM3Zf(-). The ligation mix was used to
transform E. coli XL-1 blue. Plasmid DNA was isolated from white
10 transformants and sequenced by the dideoxy chain termination method.
The base sequence is shown in Fig. 4, SEQ. ID:10:.

EXAMPLE 4

15 Assembly of complete coding region of human alpha1c adrenergic
receptor:

20 The complete coding region of human alpha1c adrenergic receptor
was assembled by ligating the cDNA clone (see Example 2, figure 3,
SEQ ID:7:) and 3'CG (see Example 3, figure 4, SEQ ID:10:) at their
common PvuII site (1552-1557 of figure 3, SEQ ID:7: and 59-64 of
figure 4, SEQ ID:10:). The complete nucleotide sequence is shown in
figure 5, SEQ ID:11:. The amino acid sequence is shown in figure 6,
SEQ. ID:12:. Figure 7 shows the structure of the cDNA, including the
25 5'-untranslated sequences. The very 3' twenty seven nucleotides (6
amino acids) shown is the sequence of the PCR primer used to generate
the sequence. However, the function of the receptor, both for ligand
binding and signal transduction depends on sequences far removed from
the carboxy terminus of the receptor. A completely human sequence is
30 shown in figure 18, SEQ. ID:26: which is truncated at the 3' terminus.

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EXAMPLE 5**Expression of the cloned alpha1C adrenergic receptor:**

5 The complete sequence (SEQ ID:11:) of the human alpha1C adrenergic receptor was subcloned into the eukaryotic expression vector pcDNA1-neo (Invitrogen). The resulting plasmid was transfected into COS-7 cells by electroporation. Cells were harvested after 72 hours and the membranes containing the expressed receptor protein were
10 prepared as described in Schwinn, et al., *J. Biol. Chem.*, 265:8183-8189, 1990. Membranes (5-25 ug, see figure 8) prepared from the COS-7 cells transfected with the vector containing the alpha1C receptor gene specifically bound the alpha 1 antagonist [¹²⁵I]-HEAT;
15 membranes prepared from the COS-7 cells transfected with the vector alone did not bind the alpha 1 antagonist [¹²⁵I]-HEAT (figure 8), proving the expression of the alpha1C adrenergic receptor. Binding reactions (total volume = 200 ul) contained 50 mM Tris-HCl pH. 7.4, 5 mM EDTA, 150 mM NaCl, 100 pM [¹²⁵I]-HEAT, and membranes prepared from COS-7 cells transfected with expression plasmids.
20 Reactions were incubated at room temperature for one hour with shaking. Reactions were filtered onto Whatman GF/C glass fiber filters with a Brandel cell harvester. Filters were washed three times with ice cold buffer and bound radioactivity was determined. Non specific
25 binding was determined in the presence of 10 uM prazosin.

30

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EXAMPLE 6**Screening assay: Alpha 1 C Adrenergic Receptor Binding**

5 Membranes prepared from the transfected COS-7 cells may also be used to identify compounds that bind to the human alpha1C adrenergic receptor. These competition binding reactions (total volume = 200 ul) contain 50 mM Tris-HCl pH. 7.4, 5 mM EDTA, 150 mM NaCl, 100 pM [125 I]-HEAT, membranes prepared from COS-7 cells transfected with the
10 alpha1C expression plasmid and increasing amounts of unlabeled ligand. Reactions are incubated at room temperature for one hour with shaking. Reactions were filtered onto Whatman GF/C glass fiber filters with a Brandel cell harvester. Filters were washed three times with ice cold buffer and bound radioactivity was determined. Binding data were analyzed and
15 IC50s determined by an iterative curve fitting program. Results are shown in Figure 9.

EXAMPLE 7

20

Expression of human alpha1A adrenergic receptor:

 The complete coding region for the human alpha1A adrenergic receptor (Bruno, et al., BBRC., 179:1485-1490, (1991); see figure 10, SEQ. ID:13: and figure 11, SEQ. ID:14: herein) was subcloned into the
25 eukaryotic expression vector pcDNA1-neo (Invitrogen). The resulting plasmid was transfected into COS-7 cells by electroporation. Cells were harvested after 72 hours and the membranes containing the expressed receptor protein were prepared as described in Schwinn, et al., J. Biol. Chem., 265:8183-8189, 1990. Membranes prepared from the COS-7
30 cells transfected with the vector containing the alpha1A receptor gene specifically bound the alpha 1 antagonist [125 I]-HEAT; membranes prepared from the COS-7 cells transfected with the vector alone did not bind the alpha 1 antagonist [125 I]-HEAT.

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Binding reactions (total volume = 200 ul) contained 50 mM Tris-HCl pH. 7.4, 5 mM EDTA, 150 mM NaCl, 100 pM [¹²⁵I]-HEAT, and membranes prepared from COS-7 cells transfected with expression plasmids. Reactions are incubated at room temperature for one hour with shaking. Reactions were filtered onto Whatman GF/C glass fiber filters with a Brandel cell harvester. Filters were washed three times with ice cold buffer and bound radioactivity was determined. Non-specific binding was determined in the presence of 10 uM prazosin.

EXAMPLE 8

Expression of human alpha1B adrenergic receptor:

1. PCR amplification of partial cDNA for human alpha1B adrenergic receptor:

Amplification of 5XB clones

5XB, SEQ. ID:15: 5' TCT AGA CCA TGA AYC CNG AYC TGG 3'

A1B, SEQ. ID:16: 5' TTT GAA TTC ACA TWC CGA CYA CAA TGC CC 3'

Oligonucleotides 5XB and A1B were used as primers in a reverse transcription PCR amplification of human heart mRNA (Clontech) using the Invitrogen Copy Kit. Briefly, 1.0 ug of mRNA was reverse transcribed in a volume of 20 ul using oligonucleotide WC' as primer.

Primary reaction (50 ul)

5 ul 10X buffer from Perkin Elmer Cetus

GeneAmp Kit

8 ul 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP

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5 2.5 ul first strand cDNA
 1 ul 25 pMoles oligo 5XB
 1 ul 25 pMoles oligo A1B
 0.25 ul 1.25 units Amplitaq DNA polymersase
 32.75 ul water

Reaction conditions; 40 cycles of 94°C 1'; 58°C 2'; 72°C 2'

10 The PCR product was directly ligated into pCR vector (Invitrogen)
and used to transform E. coli INVαF' (Invitrogen). Plasmid DNA was
isolated from white transformants and sequenced by the dideoxy chain
termination method. The base sequence is shown in Fig. 12, SEQ.
ID:17:

15 2. Amplification of EFK clones

EFK, SEQ. ID:18: 5' GAAGGCGCGCTTGAAGTC 3'

20 5B1, SEQ. ID:19: 5' AGAGAACCACCAAGAACC 3'

25 Oligonucleotides EFK and 5B1 were used as primers in a reverse
transcription PCR amplification of human aorta mRNA (Clontech)
using the Invitrogen Copy Kit. Briefly, 1.0 ug of mRNA was reverse
transcribed in a volume of 20 ul using oligo dT as primer.

Primary reaction (50 ul)

30 5 ul 10X buffer from Perkin Elmer Cetus
 GeneAmp Kit
 8 ul 1.25 mM each stock of dATP,dCTP,dGTP, and
 dTTP
 2.0 ul first strand cDNA
 1 ul 25 pMoles oligo EFK
 1 ul 25 pMoles oligo 5B1

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0.25 ul 1.25 units Amplitaq DNA polymerase
33.25 ul water

5 Reaction conditions; 40 cycles of 94°C 1'; 58°C 2'; 72°C 2'

 The PCR product was directly ligated into pCR vector (Invitrogen)
 and used to transform E. coli INVaF' (Invitrogen). Plasmid DNA was
 isolated from white transformants and sequenced by the dideoxy chain
10 termination method. The base sequence is shown in Fig. 13, SEQ.
 ID:20:.

3. Assembly of partial cDNA for human alpha1B adrenergic receptor

15 A partial cDNA clone encoding the human alpha1B adrenergic
 receptor was assembled by joining the 5XB sequence (SEQ. ID:17:) and
 the EFK sequence (SEQ. ID:20:) at their common BamHI site.

4. Amplification of the 3' end of rat alpha1B adrenergic receptor

20 S4B, SEQ. ID:21: 5' TTT GAA TTC ATG TTC AAG GTG GTG TTC
 3'

 3'B2, SEQ. ID:22: 5' TTT GAA TTC TAA AASTGN CCN GGN SCC
25 AGN GGC AT 3'

 Oligonucleotides S4B and 3'B2 were used as primers in a reverse
 transcription PCR amplification of rat heart mRNA using the Invitrogen
 Copy Kit. Briefly, 0.6 ug of mRNA was reverse transcribed in a
30 volume of 20 ul using oligo dT as primer.

 Primary reaction (50 ul)

 5 ul 10X buffer from Perkin Elmer Cetus
 GeneAmp Kit

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8 ul 1.25 mM each stock of dATP,dCTP,dGTP, and
dTTP

2.0 ul first strand cDNA

1 ul 25 pMoles oligo EFK

5 1 ul 25 pMoles oligo 5B1

0.25 ul 1.25 units Amplitaq DNA polymersase

33.25 ul water

10 Reaction conditions; 40 cycles of 94°C 1'; 58°C 2'; 72°C 2'

The PCR product was directly ligated into pCR vector (Invitrogen)
and used to transform E. coli INV α F' (Invitrogen). Plasmid DNA was
isolated from white transformants and sequenced by the dideoxy chain
15 termination method. The base sequence is shown in Fig. 14, SEQ.
ID:23:.

5. Assembly and expression of a functional human/rat hybrid alpha1B adrenergic receptor

20 The partial human alpha1B adrenergic receptor cDNA was joined
to the 3' end of the rat alpha1B adrenergic receptor cDNA at their
common BssHII restriction endonuclease site. This composite sequence
is shown in figure 15, SEQ. ID:24:, and the amino acid sequence is
shown in Fig. 16, SEQ. ID:25:

25 The complete coding region for the human/rat alpha1B
adrenergic receptor was subcloned into the eukaryotic expression vector
pcDNAI-neo (Invitrogen). The resulting plasmid was transfected into
COS-7 cells by electroporation. Cells were harvested after 72 hours
and the membranes containing the expressed receptor protein were
30 prepared as described in Schwinn, et al., J. Biol. Chem., 265:8183-
8189, 1990. Membranes prepared from the COS-7 cells transfected
with the vector containing the alpha1B receptor gene specifically bound
the alpha 1 antagonist [¹²⁵I]-HEAT; membranes prepared from the
COS-7 cells transfected with the vector alone did not bind the alpha 1

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antagonist [125 I]-HEAT. Binding reactions (total volume = 200 μ l) contained 50 mM Tris-HCl pH. 7.4, 5 mM EDTA, 150 mM NaCl, 100 pM [125 I]-HEAT, and membranes prepared from COS-7 cells transfected with expression plasmids. Reactions are incubated at room temperature for one hour with shaking. Reactions were filtered onto Whatman GF/C glass fiber filters with a Brandel cell harvester. Filters were washed three times with ice cold buffer and bound radioactivity was determined. Non specific binding was determined in the presence of 10 μ M prazosin.

EXAMPLE 9

Selective Binding assays

Membranes prepared from COS-7 cells transfected with the human alpha 1 receptor subtype expression vectors may also be used to identify compounds that selectively bind to the human alpha1C adrenergic receptor. These competition binding reactions (total volume = 200 μ l) contain 50 mM Tris-HCl pH. 7.4, 5 mM EDTA, 150 mM NaCl, 100 pM [125 I]-HEAT, membranes prepared from COS-7 cells transfected with the respective alpha 1 subtype expression plasmid and increasing amounts of unlabeled ligand. Reactions are incubated at room temperature for one hour with shaking. Reactions were filtered onto Whatman GF/C glass fiber filters with a Brandel cell harvester. Filters were washed three times with ice cold buffer and bound radioactivity was determined. Binding data were analyzed and IC50s determined by an iterative curve fitting program. Table I shows the results from such an analysis.

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	<u>Compound</u>	<u>IC₅₀ (nM)</u>		
		<u>α1A</u>	<u>α1B</u>	<u>α1C</u>
5	prazosin	0.6	2.4	1
	terazosin	4	5	19
	doxazosin	5	2	9
10	phenoxybenzamine	6.1	4.3	4.0
	WB4101	1	16	1
15	benoxathian	2.5	68	1.5
	phentolamine	36	650	14
	5-methyl urapidil	42	270	3.5
20	S(+) niguldipine	130	670	1.4

EXAMPLE 10**IDENTIFICATION AND CLONING OF A NEW ALLELE FOR THE HUMAN ALPHA1-C ADRENERGIC RECEPTOR****Probes:**

3'CG: A 525 bps fragment, specific to complete exon.2 of human α 1c AR, was PCR amplified from human genomic DNA using a sense primer based on the isolated cDNA clone and an antisense primer based on the last six amino acids of bovine α 1c cDNA. This PCR product was subcloned and confirmed by sequencing (see Example 3, SEQ.ID:10:).

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Genomic Library Screening:

Human W138 Fibroblast genomic library synthesized in the Lambda Fix II vector (2×10^6 recombinants; Stratagene, La Jolla, CA) was screened with (3'CG). This probe was labelled with ^{32}P -dCTP (Amersham) by random-primed labelling kit (Boehringer Mannheim, Indianapolis, IN). A Total 800,000 plaques were screened, using duplicate Hybond-N nylon filters (Amersham, UK). Prior to hybridization, filters were denatured (1.5M NaCl+0.5M NaOH), neutralized (1.5M NaCl+ 1M Tris.Cl, pH 8.0) and washed (0.2M Tris.Cl pH 7.5 + 2 SSC), 5' for each. DNA was cross-linked with UV cross-linker (Stratagene, La Jolla, CA). The filters were, then, hybridized in 50% formamide, 5 x SSC (1xSSC= 0.15M NaCl, 0.015M Na citrate, pH7.0), 0.02% polyvinylpyrophosphate, 0.2% Ficoll, 0.2% bovine serum albumin, 150 μg of sheared & boiled Salmon sperm DNA, 10 6 cpm of ^{32}P -labelled probe at 42°C for 40hrs. Filters were washed in 0.1x SSC +1% SDS solution at 60°C for 20'. Two more rounds of screening for 20 "positive" plaques/clones with 3'CG probe confirmed two clones for the alpha1C adrenergic receptor, which were named 48.1C and 53.1C. Clone 53.1C was subjected to further analysis/investigation.

Sub-clonning of Exon.2 :

53.1C lambda DNA was amplified by plate lysis method and purified with Qiagen midi-lambda kit (Qiagen, Chatsworth, CA). A 2.6Kb band excised with EcoRI restriction enzyme was identified by Southern analysis using 3'CG probe. This fragment was then subcloned into pGEM3Zf(+) vector.

DNA sequencing:

Nucleotide sequence analysis of DNA in both direction was performed by Sanger chain termination method.

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Result and Discussion:

Sequencing analysis of this genomic clone confirmed that clone 53.1c contains sequences for complete exon.2 flanked by an intron at 5'-end. It also reveals that there is a nucleotide change from cytosine (C) to thymine(T) at nucleotide position 1636, amino acid position 347. This change creates a PstI site and changes the codon for arginine (Arg) to cystine (Cys). This data differs from the known/published cDNA sequence of the gene. Southern analysis of human genomic DNA confirms the PstI site in the gene/exon.2.

EXAMPLE 11**COMPARATIVE PHARMACOLOGY OF ALPHA1-C ALLELES**

We have cloned two genes for the human alpha-1c receptor. The coding regions differ by a single nucleotide. The genes encode either Cys or Arg at amino acid 347 near the C terminus of the receptor. The nucleotide difference lies within a PstI restriction enzyme recognition site thus creating a Restriction Fragment Length Polymorphism (RFLP). The frequency of allele 1 (LRR) is 0.34; allele 2 (LCR) is 0.66 in 83 unrelated individuals (Hoehe et al "A two-allele PstI RFLP for the alpha-1C adrenergic receptor gene" Human Molecular Genetics 1: 349, 1992; Allele 1 is defined by a 2.1 kb PstI fragment; allele 2 yields two bands of 1.6 and 0.5 kb). Since the amino acid difference occurs within the intracellular tail of the receptor we would not expect any pharmacological differences between the expressed receptors. To investigate the pharmacological profiles of the two allelic forms of the human alpha-1c adrenergic receptor we ligated the genomic exon 2 fragment of allele 2 to a cDNA clone of allele 1 at a common PvuII restriction site. The two allelic forms were transiently expressed in COS-7 cells using pcDNA1/NEO (Invitrogen) expression vector. Competitive inhibition studies performed in the presence of ¹²⁵I-HEAT with various antagonists showed no significant difference in their pharmacological profiles (Table II):

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Table II
COMPARATIVE PHARMACOLOGY OF ALPHA1-C ALLELES

	<u>IC₅₀ (nM)</u>	
	<u>LRR</u>	<u>LCR</u>
5 phentolamine	15	17
niguldipine	0.8	1.8
prazosin	1.0	0.9
5-methyl urapidi	3.1	4.3
10 WB4101	0.9	1.0

EXAMPLE 12

15 CLONING OF A NOVEL ALPHA1-A ADRENERGIC RECEPTOR

A cosmid library containing FG293 cell line genomic DNA in the double-cos vector sCos-1 was screened as follows: The published human α_1a receptor cDNA clone (Bruno et al., BBRC, 179:1485-1490 (1991), and see Fig. 10, SEQ.ID:13:) was cloned into the vector
 20 pcDNA1 neo to generate the clone pEX α_1a . Filters containing approximately 200,000 clones were screened by colony hybridization ([Sambrook, *Molecular Cloning*, Cold Spring Harbor Laboratory Press, New York, 1989]) using a mixed exon 1 probe generated by PCR
 25 corresponding to α_1a (TMD1-3), α_1b (TMD1-5) and α_1c (TMD1-5): 25 cycles of 95° C 1'; 52 °C 30 sec; 72 °C 1.5' using 10 ng of pEX α_1b , pEX α_1c or pEX α_1a and 10 pmoles each of primers 5' MET (5' GAATCCCGACCTGGAC), SEQ.ID:31:, and 3' BAM (5'GGATCCTCAGGGTC), SEQ.ID:32:, for α_1b , 5' 597 (5' CCATGGTGTCTCTCTCGGG), SEQ.ID:33: and 3' 1219 (5' GACGCGGCAGTACATGAC), SEQ.ID:34: for α_1c or 5' 76 (5' GTCATGATGGCTGGGTACTTG), SEQ.ID:35:, for α_1a in a 12 μ l reaction containing 1.5 μ M each unlabelled dNTP and 50 μ Ci 3000 Ci/mmol α -[³²P] dCTP. The filters were incubated with 1 x 10⁶ cpm/ml of probe in 5X SSC, 35% Formamide, 0.02% SDS, 0.1 %

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lauroyl sarcosine, 2% blocking buffer (Boehringer Mannheim), at 42 °C for 18 hours. The filters were washed with 2 liters of 0.5X SSC, 0.1% SDS, 55 °C and exposed to Kodak XAR-5 film. Twelve primary positives were picked from master plates and re-screened using the α 1a-specific probe. Cosmid DNA was prepared from second round positive clones, digested with endonucleases Eco RI or Hind III and subjected to Southern blot analysis: Fragments were resolved by electrophoresis, and transferred to a nitrocellulose membrane (Boehringer Mannheim) with 20X SSC (1X SSC = 0.15M Sodium chloride, 0.015M Sodium citrate, pH 7.0) according to the method of Southern ([Southern, 1975 #14]). The membrane was hybridized, washed and analyzed as described above. Alpha-1a, α 1b, and α 1c receptor clones were identified by comparison of restriction patterns with genomic southern blots performed with α 1a, α 1b, or α 1c specific probes. A Cosmid containing α 1a receptor exon 1 DNA was subjected to restriction digestion by endonuclease Pst I and subjected to southern blot analysis as above using the α 1a -specific probe. Two fragments of 2.3 and 1.6 kb were detected and subcloned into the Pst I site of PGEM 3ZF. The presence of the correct 5' terminal sequences in the 2.3 kb fragment was confirmed by sequencing across the junction between inverted repeat and non-repeat sequences. The 5' end of the α 1a receptor gene was ligated to the cDNA clone at their common PstI site, see figures 22-24, SEQ.ID:29:, and SEQ.ID:30:.

EXAMPLE 13

EXEMPLARY COUNTERSCREENS

1. Assay Title: Dopamine D2,D4 in vitro screen

Objective of the Assay:

The objective of this assay is to eliminate agents which specifically affect binding of [3H] spiperone to cells expressing human dopamine receptors D2, D3 or D4.

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Method:

Modified from VanTol et al (1991); Nature (Vol 350) Pg 610-613.

5 Frozen pellets containing specific dopamine receptor subtypes stably expressed in clonal cell lines are lysed in 2 ml lysing buffer (10mM Tris-HCl/5mM Mg, pH 7.4). Pellets obtained after centrifuging these membranes (15' at 24,450 rpm) are resuspended in 50mM Tris-HCl pH 7.4 containing EDTA, MgCl₂, KCl, NaCl, CaCl₂ and ascorbate to give a 1 Mg/mL suspension. The assay is initiated by
10 adding 50-75 µg membranes in a total volume of 500 µl containing 0.2 nM [3H]-spiperone. Non-specific binding is defined using 10 µM apomorphine. The assay is terminated after a 2 hour incubation at room temperature by rapid filtration over GF/B filters presoaked in
15 0.3% PEI, using 50mM Tris-HCl pH 7.4.

2. Assay Title: Serotonin 5HT1a**Objective of the Assay**

20 The objective of this assay is to eliminate agents which specifically affect binding to cloned human 5HT1a receptor

Method:

Modified from Schelegel and Peroutka *Biochemical Pharmacology* 35: 1943-1949 (1986).
25

Mammalian cells expressing cloned human 5HT1a receptors are lysed in ice-cold 5 mM Tris-HCl, 2 mM EDTA (pH 7.4) and homogenized with a polytron homogenizer. The homogenate is centrifuged at 1000Xg for 30', and then the supernatant is centrifuged again at 38,000Xg for 30'. The binding assay contains 0.25 nM [3H]8-OH-DPAT in 50 mM Tris-HCl, 4 mM CaCl₂ and 1mg/ml ascorbate.
30 Non-specific binding is defined using 10 µM propranolol. The assay is terminated after a 1 hour incubation at room temperature by rapid filtration over GF/C filters

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EXAMPLE 14
EXEMPLARY FUNCTIONAL ASSAYS

5 In order to confirm the specificity of compounds for the human $\alpha 1C$ adrenergic receptor and to define the biological activity of the compounds, the following functional tests may be performed:

10 1. **IN VITRO RAT, DOG AND HUMAN PROSTATE**
 AND DOG URETHRA

 Taconic Farms Sprague-Dawley male rats, weighing 250-400 grams are sacrificed by cervical dislocation under anesthesia (methohexital; 50 mg/kg, i.p.). An incision is made into the lower
15 abdomen to remove the ventral lobes of the prostate. Each prostate removed from a mongrel dog is cut into 6-8 pieces longitudinally along the urethra opening and stored in ice-cold oxygenated Krebs solution overnight before use if necessary. Dog urethra proximal to prostate is cut into approximately 5 mm rings, the rings are then cut open for
20 contractile measurement of circular muscles. Human prostate chips from transurethral surgery of benign prostate hyperplasia are also stored overnight in ice-cold Krebs solution if needed.

 The tissue is placed in a Petri dish containing oxygenated Krebs solution [NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4 , 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 2.0 mM; dextrose, 11
25 mM] warmed to 37°C. Excess lipid material and connective tissue are carefully removed. Tissue segments are attached to glass tissue holders with 4-0 surgical silk and placed in a 5 ml jacketed tissue bath containing Krebs buffer at 37°C, bubbled with 5% $CO_2/95\% O_2$. The
30 tissues are connected to a Statham-Gould force transducer; 1 gram (rat, human) or 1.5 gram (dog) of tension is applied and the tissues are allowed to equilibrate for one hour. Contractions are recorded on a Hewlett-Packard 7700 series strip chart recorder.

 After a single priming dose of 3 μM (for rat), 10 μM (for dog) and 20 μM (for human) of phenylephrine, a cumulative

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concentration response curve to an agonist is generated; the tissues are washed every 10 minutes for one hour. Vehicle or antagonist is added to the bath and allowed to incubate for one hour, then another cumulative concentration response curve to the agonist is generated.

EC50 values are calculated for each group using GraphPad Inplot software. pA_2 ($-\log K_b$) values were obtained from Schild plot when three or more concentrations were tested. When less than three concentrations of antagonist are tested, K_b values are calculated according to the following formula $K_b = \frac{[B]}{x-1}$,

where x is the ratio of EC50 of agonist in the presence and absence of antagonist and $[B]$ is the antagonist concentration.

2. MEASUREMENT OF INTRA-URETHRAL PRESSURE IN ANESTHETIZED DOGS

PURPOSE: Benign prostatic hyperplasia causes decreased urine flow rate that may be produced by both passive physical obstruction of the prostatic urethra from increased prostate mass as well as active obstruction due to prostatic contraction. Alpha adrenergic receptor antagonists such as prazosin and terazosin prevent active prostatic contraction, thus improve urine flow rate and provide symptomatic relief in man. However, these are non-selective alpha-1 receptor antagonists which also have pronounced vascular effects. Because we have identified the alpha-1C receptor subtype as the predominant subtype in the human prostate, it is now possible to specifically target this receptor to inhibit prostatic contraction without concomitant changes in the vasculature. The following model is used to measure adrenergically mediated changes in intra-urethral pressure and arterial pressure in anesthetized dogs in order to evaluate the efficacy and potency of selective alpha adrenergic receptor antagonists. The goals are to: 1) identify the alpha-1 receptor subtypes responsible for prostatic/urethral contraction and vascular responses, and 2) use this model to evaluate novel selective alpha adrenergic antagonists. Novel

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and standard alpha adrenergic antagonists may be evaluated in this manner.

5 **METHODS:** Male mongrel dogs (7-12 kg) are used in this study. The dogs are anesthetized with pentobarbital sodium (35 mg/kg, i.v. plus 4 mg/kg/hr iv infusion). An endotracheal tube is inserted and the animal ventilated with room air using a Harvard instruments positive displacement large animal ventilator. Catheters (PE 240 or 260) are
10 placed in the aorta via the femoral artery and vena cava via the femoral veins (2 catheters, one in each vein) for the measurement of arterial pressure and the administration of drugs, respectively. A supra-pubic incision ~1/2 inch lateral to the penis is made to expose the urethers, bladder and urethra. The urethers are ligated and cannulated so that
15 urine flows freely into beakers. The dome of the bladder is retracted to facilitate dissection of the proximal and distal urethra. Umbilical tape is passed beneath the urethra at the bladder neck and another piece of umbilical tape is placed under the distal urethra approximately 1-2 cm distal to the prostate. The bladder is incised and a Millar micro-tip pressure transducer is advanced into the urethra. The bladder incision
20 is sutured with 2-0 or 3-0 silk (purse-string suture) to hold the transducer. The tip of the transducer is placed in the prostatic urethra and the position of the Millar catheter is verified by gently squeezing the prostate and noting the large change in urethral pressure.

25 Phenylephrine, an alpha-1 adrenergic agonist, is administered (0.1-100 ug/kg, iv; 0.05 ml/kg volume) in order to construct dose response curves for changes in intra-urethral and arterial pressure. Following administration of increasing doses of an alpha adrenergic antagonist (or vehicle), the effects of phenylephrine on
30 arterial pressure and intra-urethral pressure are re-evaluated. Four or five phenylephrine dose-response curves are generated in each animal (one control, three or four doses of antagonist or vehicle). The relative antagonist potency on phenylephrine induced changes in arterial and intra-urethral pressure are determined by Schild analysis. The family of averaged curves are fit simultaneously (using ALLFIT software

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package) with a four parameter logistic equation constraining the slope, minimum response, and maximum response to be constant among curves. The dose ratios for the antagonist doses (rightward shift in the dose-response curves from control) are calculated as the ratio of the ED₅₀'s for the respective curves. These dose-ratios are then used to construct a Schild plot and the K_b (expressed as ug/kg, iv) determined. The K_b (dose of antagonist causing a 2-fold rightward shift of the phenylephrine dose-response curve) is used to compare the relative potency of the antagonists on inhibiting phenylephrine responses for intra-urethral and arterial pressure. The relative selectivity is calculated as the ratio of arterial pressure and intra-urethral pressure K_b's. Effects of the alpha-1 antagonists on baseline arterial pressure are also monitored. Comparison of the relative antagonist potency on changes in arterial pressure and intra-urethral pressure provide insight as to whether the alpha receptor subtype responsible for increasing intra-urethral pressure is also present in the systemic vasculature. According to this method, one is able to confirm the selectivity of alpha_{1C} adrenergic receptor antagonists that prevent the increase in intra-urethral pressure to phenylephrine without any activity at the vasculature.

At the end of the experiment, the dogs are killed via an overdose of intravenously administered pentobarbital or saturated KCl.

25

EXAMPLE 15

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

30 Step 1: A mixture of 15.4 g 4-piperidone hydrochloride hydrate, 200 mL ether, 100 mL saturated aqueous Na₂CO₃ solution and 21.8 g di-t-butylidicarbonate was vigorously stirred for 48 h. The layers were separated and the organic layer was washed with two 150 mL portions of 10% aqueous citric acid, dried over MgSO₄. Removal of solvents

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under reduced pressure gave 18.9 g (95%) N-t-butyloxycarbonyl-4-piperidone as a white solid.

5 Step 2: A mixture of 6.0 g N-t-butyloxycarbonyl-4-piperidone, 3.3 g of 2-aminophenol, 25 mL of 1,2-dichloroethane, 25 mL of glacial acetic acid, and 500 mg powdered 4Å molecular sieves was stirred under inert atmosphere. After 30 min, 6.4 g sodium triacetoxymethylborohydride was added stirring was continued for 38 h. The reaction mixture was poured into 400 mL ethyl acetate and 200 mL saturated aqueous NaHCO₃ and the layers separated. The organic layer was washed with brine (2 x 100 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. Chromatography of the crude product on silica gel, eluting with a gradient of 1-3% methanol/methylene chloride containing 0.5% concentrated NH₄OH gave 6.95 g (79%) 1-t-butyloxycarbonyl-4-(2-hydroxyphenylamino)piperidine as an orange foam.

15 Step 3: To a stirred solution of 6.95 g 1-t-butyloxycarbonyl-4-(2-hydroxyphenylamino)piperidine and 6.2 mL diisopropylethylamine in 120 mL tetrahydrofuran cooled to 0°C was added 3.0 g triphosgene. The reaction was stirred 30 min at 0°C and then at room temperature 20 2 h. The precipitate was removed by filtration, the filtrate concentrated at reduced pressure and partitioned between 250 mL ethyl acetate and 100 mL saturated aqueous Na₂CO₃. The layers were separated, the organic layer washed with 100 mL of saturated aqueous Na₂CO₃, 100 mL of water, 100 mL of brine, dried over MgSO₄, and 25 concentrated under reduced pressure. Chromatography of the crude product on silica gel, eluting with a gradient of 40-50% ethyl acetate-hexanes gave 6.0 g (79%) 1-((1-t-butyloxycarbonyl)piperidin-4-yl)-3-benzoxazolin-2-one as a yellow foam.

30 Step 4: A stirred solution of 6.0 g (19 mmol) 1-((1-t-butyloxycarbonyl)piperidin-4-yl)-3-benzoxazolin-2-one in 120 mL ethyl acetate was cooled to -78°C and a stream of hydrogen chloride gas was introduced with a fritted dispersion tube for 15 min. The mixture was allowed to warm to 0°C for 1 h, then room temperature for 2 h. The resulting precipitate was collected by filtration. Drying

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at reduced pressure for 8 h gave 4.2 g (16.5 mmol, 88%) of the hydrochloride salt of 1-(4-piperidinyl)-3-benzoxazolin-2-one as an off-white solid.

5 Step 5: A mixture of 430 mg (1.7 mmol) of the hydrochloride salt of 1-(4-piperidinyl)-3-benzoxazolin-2-one and 588 mg (1.9 mmol) of 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one, 20 mL dry dimethylformamide and 0.6 mL (3.5 mmol) diisopropylethylamine was warmed to 80°C for 3 h. The solvents were removed under
10 reduced pressure and the crude oily product dissolved in 100 mL ethyl acetate, washed with saturated aqueous NaHCO₃ (3 x 30 mL), brine (1 x 30 mL), dried over Na₂SO₄ and concentrated at reduced pressure. Chromatography of the crude product on silica gel, eluting with a gradient of 10% methanol in methylene chloride and recrystallization from ethyl acetate gave 300 mg (40%) of 1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one
15 as an off-white crystalline solid. Analysis calculated for C₂₃H₂₅N₃O₅S C: 60.64, H: 5.53, N: 9.22 found C: 60.04, H: 5.50, N: 9.29. ¹H NMR (300 MHz, CDCl₃) 8.07 (dd, J = 6.54, 2.2 Hz, 1H), 7.93 (m, 1H), 7.85 (m, 2H), 7.18 (m, 4H), 4.21 (tt, J = 12.4, 4.34 Hz, 1H), 3.85 (t, J = 7.27 Hz, 2H), 3.07 (d, J = 11.67 Hz, 2H), 2.45 (t, J = 7.57 Hz, 2H), 2.35 (dt, J = 12.45, 3.71 Hz, 2H), 2.11 (dt, J = 12.1, 1.84 Hz, 2H), 1.90 (m, 4H), 1.64 (m, 2H).
20

EXAMPLE 16

25 **1,1-Dioxido-2-(4-(4-(3,4-dihydro-2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

From the hydrochloride salt of 3,4-dihydro-1-(4-piperidyl)-2(1H)-quinolinone prepared according to H. Ogawa et. al. *J. Med. Chem.*
30 **1993, 36, 2011-2017**, and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained 1,1-dioxido-2-(4-(4-(3,4-dihydro-2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

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as a white solid: ^1H NMR (300 MHz, CDCl_3) 8.07 (dd, $J = 6.5, 2.3$ Hz, 1H), 7.92 (t, $J = 6.73$ Hz, 1H), 7.84 (m, 2H), 7.21 (t, $J = 8.73$ Hz, 2H), 7.19 (m, 1H), 6.99 (t, $J = 7.13$ Hz, 1H), 4.35 (tt, $J = 12.2, 4.2$ Hz, 1H), 3.83 (t, $J = 7.32$ Hz, 2H), 3.04 (d, $J = 11.42$ Hz, 2H), 2.81 (t, $J = 6.59$ Hz, 2H), 2.61 (m, 3H), 2.43 (t, $J = 7.19$ Hz, 2H), 2.09 (t, $J = 11.71$ Hz, 2H), 1.88 (m, 2H), 1.69 (m, 5H).

EXAMPLE 17

1,1-Dioxido-2-(4-(4-(1,2-dihydro-4(H)-2-oxo-3,1-benzoxazinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

Step 1: A mixture of 20 g of N-t-butyloxycarbonyl-4-piperidinone, 13 g of 2-aminobenzyl alcohol 14 mL of acetic acid and 500 mL of toluene was refluxed under inert atmosphere with azeotropic removal of water for 16 h. After cooling to ambient temperature, 14 g of sodium cyanoborohydride and 200 mL tetrahydrofuran were added and the mixture was stirred at ambient temperature for 24 h. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in 750 mL of ethyl acetate and washed with saturated aqueous NaHCO_3 (4x 500 mL) and brine (250 mL). The organic layer was dried over MgSO_4 , and the solvent was removed under reduced pressure. Chromatography of the crude product on silica gel, eluting with a gradient of 15-30% ethyl acetate-hexanes gave 24 g (78%) of 1-t-butyloxycarbonyl-4-((2-hydroxymethyl)phenylamino)piperidine as a gum.

Step 2. To a stirred mixture of 24 g of 1-t-butyloxycarbonyl-4-((2-hydroxymethyl)-phenylamino)piperidine, 250 mL of tetrahydrofuran, 41 mL of diisopropylethylamine cooled to 0°C was added 8.54 g of triphosgene. The reaction was stirred at 0°C for 1h, and then at ambient temperature for 72 h. Ether (250 mL) was added, the mixture was cooled to 0°C for 3 h, the precipitate removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in 750 mL of ethyl acetate, washed with 5% aqueous citric

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acid (2x 500 mL), water (250 mL), saturated aqueous NaHCO₃ (2x 500 mL), dried over MgSO₄ and concentrated under reduced pressure.

The residue was boiled in ether (ca. 200 mL) until the solid had dissolved. Cooling overnight gave 19 g of 1-((1-t-butylloxycarbonyl)piperidin-4-yl)-1,2-dihydro-4(H)-3,1-benzoxazin-2-one as off-white crystals (75% yield).

Step 4. A stream of hydrogen chloride gas was dispersed through a stirred, ice cold solution of 19 g of 1-((1-t-butylloxycarbonyl)piperidin-4-yl)-1,2-dihydro-4(H)-3,1-benzoxazin-2-one in 500 mL of ethyl acetate for 30 min. Stirring was continued at 0°C for 1 h, then at ambient temperature for 1 h. The suspension was diluted with 250 mL of ether, aged for 1 h at 0°C, and the solid product was collected by filtration. Drying under reduced pressure for 18 h, gave 14 g of the hydrochloride salt of 1-(4-piperidinyl)-1,2-dihydro-4(H)-3,1-benzoxazin-2-one as an off-white solid (91%).

Step 5: From the hydrochloride salt of 1-(4-piperidinyl)-1,2-dihydro-4(H)-3,1-benzoxazin-2-one, and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.10 (t, J = 7.33 Hz, 1H), 7.99 (m, 3H), 7.42 (t, J = 7.14 Hz, 1H), 7.27 (t, J = 7.51 Hz, 2H), 7.16 (t, J = 7.69 Hz, 1H), 5.17 (s, 2H), 4.27 (t, J = 12.08 Hz, 1H), 3.87 (t, J = 6.22 ppm, 2H), 3.67 (m, 3H), 3.19 (m, 3H), 2.98 (m, 2H), 2.16 (d, J = 12.5 Hz, 2H), 1.92 (m, 4H).

EXAMPLE 18

1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

From the hydrochloride salt of 1-(4-piperidyl)-2(1H)-quinolinone was prepared according to H. Ogawa et. al. *J. Med. Chem.* **1993**, 36, 2011-2017, and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-

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3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃) 8.08 (d, J = 6.73 Hz, 1H), 7.90 (m, 3H), 7.7 (d, J = 9.5 Hz, 1H), 7.6 (d, J = 9.2 Hz, 1H), 7.56 (d, J = 7.62 Hz, 1H), 7.26 (m, 2H), 6.71 (d, J = 9.47 Hz, 1H), 3.85 (br m, 3H), 3.45 (br m, 1H), 3.25 (br m, 3H), 3.14 (br m, 6H), 1.95 (br s, 4H).

EXAMPLE 19

1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one

From the hydrochloride salt of 1-(4-piperidyl)-2(1H)-quinolinone prepared according to H. Ogawa et. al. *J. Med. Chem.* **1993**, 36, 2011-2017, and 2-(4-bromobutyl)-5-nitro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃) 8.88 (d, J = 1.9 Hz, 1H), 8.73 (dd, J = 8.36, 1.71 Hz, 1H), 8.15 (d, J = 8.36 Hz, 1H), 7.79 (br m, 1H), 7.62 (d, J = 3.47 Hz, 1H), 7.51 (m, 2H), 7.20 (m, 2H), 6.66 (d, J = 9.33 Hz, 1H), 3.91 (t, J = 7.33 Hz, 2H), 2.85 (br m, 2H), 2.48 (t, J = 8.27 Hz, 2H), 2.21 (t, J = 10.65 Hz, 2H).

EXAMPLE 20

6-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

From the hydrochloride salt of 1-(4-piperidyl)-2(1H)-quinolinone prepared according to H. Ogawa et. al. *J. Med. Chem.* **1993**, 36, 2011-2017, and 2-(4-bromobutyl)-6-chloro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (300MHz, CD₃OD) 8.25 (d, J = 1.66 Hz, 1H), 8.08 (d, J = 8.17 Hz, 1H), 7.98 (dd, J = 8.24, 1.71 Hz, 1H), 7.89 (d, J = 9.52 Hz, 1H), 7.85 (d, J = 8.55 Hz, 1H), 7.71 (d, J = 7.68 Hz, 1H), 7.68 (t, J = 7.57 Hz,

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1H), 7.33 (t, J = 7.65 Hz, 1H), 6.59 (d, J = 9.52 Hz, 1H), 5.06 (br m, 1H), 3.88 (t, J = 6.3 Hz, 2H), 3.69 (d, J = 9.87 Hz, 2H), 3.26 (m, 6H), 1.94 (m, 6H).

5

EXAMPLE 21

5-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

10 From the hydrochloride salt of 1-(4-piperidiny1)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-6-chloro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (300 MHz, CD₃OD) 8.11 (d, J = 7.81 Hz, 1H), 8.13 (s, 1H), 8.02 (dd, J = 8.3, 1.7 Hz, 1H), 7.90 (d, J = 9.52 Hz, 1H), 7.85 (d, J = 8.85 Hz, 1H), 7.71 (d, J = 7.57
15 Hz, 1H), 7.67 (t, J = 8.97 Hz, 1H), 7.33 (t, J = 7.32 Hz, 1H), 6.59 (d, J = 9.47 Hz, 1H), 5.08 (br m, 1H), 3.89 (t, J = 6.11 Hz, 2H), 3.71 (d, J = 8.74 Hz, 2H), 3.30 (br m, 6H), 2.0-1.9 (m, 6H).

20

EXAMPLE 22

1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-7-nitro-1,2-benzisothiazol-3(2H)-one

25 From the hydrochloride salt of 1-(4-piperidiny1)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-6-nitro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃) 8.65 (d, J = 8.19 Hz, 1H), 8.44 (d, J = 7.63 Hz, 1H), 8.10 (t, J = 8.11 Hz, 1H), 7.81 (br s, 1H), 7.61 (d, J = 9.34 Hz, 1H), 7.52 (m, 2H), 7.19 (t, J = 7.33
30 Hz, 1H), 6.67 (d, J = 9.28 Hz, 1H), 3.95 (t, J = 7.38 Hz, 2H), 3.10 (d, J = 10.37 Hz, 2H), 2.86 (br s, 2H), 2.49 (t, J = 6.95 Hz, 2H), 2.22 (t, J = 10.74 Hz, 2H), 1.98 (m, 2H), 1.70 (br m, 5H).

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EXAMPLE 23**1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one**

5 From the hydrochloride salt of 1-(4-piperidyl)-2(1H)-quinolinone prepared according to H. Ogawa et. al. *J. Med. Chem.* **1993**, 36, 2011-2017, and 2-(4-bromobutyl)-4-methoxy-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example
10 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃) 7.80 (t, J = 8.1 Hz, 2H), 7.60 (d, J = 9.33 Hz, 2H), 7.51 (m, 2H), 7.25 (m, 3H), 6.66 (d, J = 9.27 Hz, 1H), 4.06 (s, 2H), 3.80 (t, J = 6.9 Hz, 2H), 3.09 (dd, J = 9.8, 1.8 Hz, 2H), 2.84 (m, 2H), 2.46 (t, J = 7.2 Hz, 2H), 2.19 (t, J = 11.6 Hz, 2H), 1.89 (br m, 2H), 1.69 (br m, 3H), 1.58 (s, 3H).
15

EXAMPLE 24**1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one**

20 From the hydrochloride salt of 1-(4-piperidiny)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-5-nitro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (300 MHz, CD₃OD)
25 8.82 (m, 2H), 8.39 (d, J = 8.98 Hz, 1H), 7.25 (m, 4H), 4.50 (tt, J = 8.01, 3.96 Hz, 1H), 3.93 (t, J = 6.05 Hz, 2H), 3.74 (d, J = 12.94 Hz, 2H), 3.26 (m, 4H), 2.73 (m, 2H), 2.17 (d, J = 13.9 Hz, 2H), 1.93 (br m, 4H).
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EXAMPLE 25**6-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

5 From the hydrochloride salt of 1-(4-piperidiny)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-6-chloro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (300 MHz, CD₃OD)

10 8.22 (d, J = 1.41 Hz, 1H), 8.07 (dd, J = 8.3, 2.63 Hz, 1H), 7.96 (dd, J = 8.19, 1.65 Hz), 7.33 (d, J = 7.13 Hz, 1H), 7.23 (m, 2H), 4.49 (tt, J = 12.21, 4.21 Hz, 1H), 3.88 (t, J = 5.86 Hz, 2H), 3.74 (d, J = 11.72 Hz, 2H), 3.23 (br m, 4H), 2.73 (m, 2H), 2.17 (d, J = 13.43 Hz, 2H), 1.9 (br m, 4H).

15

EXAMPLE 26**5-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

20 From the hydrochloride salt of 1-(4-piperidiny)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-5-chloro-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (300 MHz, CD₃OD)

25 8.10 (d, J = 6.59 Hz, 1H), 8.11 (s, 1H), 7.29 (m, 2H), 7.21 (m, 2H), 4.49 (tt, J = 12.21, 4.21 Hz, 1H), 3.88 (t, J = 5.86 Hz, 2H), 3.74 (d, J = 11.72 Hz, 2H), 3.23 (br m, 4H), 2.73 (m, 2H), 2.17 (br d, 2H), 1.9 (br m, 4H).

EXAMPLE 27

30 **1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-7-nitro-1,2-benzisothiazol-3(2H)-one**

From the hydrochloride salt of 1-(4-piperidiny)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-7-nitro-1,1-dioxido-1,2-benzothiazol-

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3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (300 MHz, CD₃OD) 8.76 (d, J = 8.25 Hz, 1H), 8.50 (d, J = 7.76 Hz, 1H), 8.22 (t, J = 7.76 Hz), 7.24 (m, 4H), 4.49 (tt, J = 12.21, 4.21 Hz, 1H), 3.96 (t, J = 6.01 Hz, 2H), 3.76 (d, J = 11.7 Hz, 2H), 3.23 (m, 4H), 2.73 (m, 2H), 2.17 (dd, J = 14.5, 2.2 Hz, 2H), 1.9 (m, 4H).

EXAMPLE 28

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one

From the hydrochloride salt of 1-(4-piperidiny)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-4-methoxy-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃) 7.83 (t, J = 8.06 Hz, 1H), 7.49 (d, J = 7.69 Hz, 1H), 7.46 (d, J = 7.51 Hz, 1H), 7.30 (d, J = 8.6 Hz, 1H), 7.17 (m, 3H), 4.56 (dt, J = 11.72, 4.21 Hz, 1H), 4.07 (s, 3H) 3.79 (m, 4H), 3.16 (m, 2H), 2.88 (m, 4H), 2.07 (d, J = 13.0 Hz, 2H), 1.96 (m, 4H).

EXAMPLE 29

1,1-Dioxido-2-(4-(4-(5-chloro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

From the hydrochloride salt of 5-chloro-1-(4-piperidiny)-3-benzoxazolin-2-one and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.10 (td, J = 6.0, 1.2 Hz, 2H), 8.02-7.94 (overlapping dt, 2H), 7.43 (d, J = 1.8 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.16 (dd, J = 8.6, 2.0 Hz, 1H), 4.43 (tt, J = 12.4, 4.4, 1H), 3.88 (t, J = 6.4, 2H), 3.66 (d, J = 11.5, 2H), 3.16-3.05 (m, 4H), 2.66 (qd, J = 13.0, 3.7, 2H), 2.13 (d, J = 13.4, 2H), 2.01-1.88 (m, 4H).

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EXAMPLE 30

1,1-Dioxido-2-(4-(4-(3a-(R)-8a-(S)-2-oxo-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

5

From the hydrochloride salt of 4-(3a-(R)-8a-(S)-2-oxo-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazolinyl)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.10-8.07 (m, 1H), 8.02-7.94 (m, 2H), 7.59 (d, J = 7.1 Hz, 1H), 7.32-7.31 (m, 4H), 5.37-5.30 (m, 2H), 3.86 (m, 3H), 3.55-3.41 (m, 4H), 3.25-2.98 (m, 5H), 2.55 (m, 2H), 2.14 (m, 1H), 1.94-1.77 (m, 4H),

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EXAMPLE 31

1,1-Dioxido-2-(4-(4-(2-oxonaphth[2,3-d]oxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

20

From the hydrochloride salt of 4-(2-oxonaphth[2,3-d]oxazolinyl)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.11 (td, J = 6.5, 0.9 Hz, 2H), 8.02-7.94 (m, 2H), 7.89 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 5.3 Hz, 2H), 7.50-7.42 (m, 2H), 4.58 (tt, J = 12.3, 4.0 Hz, 1H), 3.90 (t, J = 6.4 Hz, 2H), 3.72 (d, J = 13.0 Hz, 2H), 3.22-3.15 (m, 4H), 2.80 (qd, J = 12.8, 3.0 Hz, 2H), 2.21 (d, J = 13.7 Hz, 2H), 2.00-1.92 (m, 4H).

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EXAMPLE 32**1,1-Dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

5 From the hydrochloride salt of 4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.09 (td, J = 5.7, 1.5 Hz, 2H), 8.03-7.94 (m, 2H), 7.21 (d, J = 8.1 Hz, 1H),
10 7.11 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 4.46 (tt, J = 12.2, 4.0 Hz, 1H), 3.88 (t, J = 6.4 Hz, 2H), 3.71 (d, J = 12.5 Hz, 2H), 3.26-3.16 (m, 4H), 2.71 (qd, J = 13.5, 3.5 Hz, 2H), 2.38 (s, 3H), 2.14 (d, J = 14.1 Hz, 2H), 1.97-1.91 (m, 4H).

15

EXAMPLE 33**1,1-Dioxido-2-(4-(4-(2-oxo-5-phenyl-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

20 From the hydrochloride salt of 4-(2-oxo-5-phenyl-3-benzoxazolinyl)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.11-8.07 (overlapping t, 2H), 8.02-7.94 (m, 2H), 7.64 (d, J = 7.1 Hz, 2H),
25 7.58 (d, J = 1.5 Hz, 1H), 7.47-7.41 (m, 3H), 7.38-7.33 (m, 2H), 4.57 (tt J = 12.4, 4.2 Hz, 1H), 3.88 (t, J = 6.4 Hz, 2H), 3.71 (d, J = 12.1 Hz, 2H), 3.23-3.13 (m, 4H), 2.77 (qd, J = 14.0, 3.0 Hz, 2H), 2.18 (d, J = 13.9, 2H), 1.97-1.90 (m, 4H).

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EXAMPLE 34

1,1-Dioxido-2-(4-(4-(6-methoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

5 From the hydrochloride salt of 4-(6-methoxy-2-oxo-3-benzoxazoliny)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400
10 MHz, CD₃OD) 8.14 (td, J = 6.4, 1.4 Hz, 2H), 8.05-8.00 (overlapping td, 2H), 7.26 (d, J = 8.6 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 6.86 (dd, J = 8.8, 2.4 Hz, 1H), 4.48 (tt, J = 12.1, 4.2 Hz, 1H), 3.92 (t, J = 6.2 Hz, 2H), 3.83 (s, 3H), 3.75 (d, J = 12.1, 2H), 3.34-3.22 (m, 4H), 2.74 (qd, J = 12.6, 3.6 Hz, 2H), 2.19 (d, J = 13.5 Hz, 2H), 2.00-1.95 (m, 4H).

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EXAMPLE 35

1,1-Dioxido-2-(4-(4-(6-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

20

From the hydrochloride salt of 4-(6-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400
25 MHz, CD₃OD) 8.01 (t, J = 6.8 Hz, 2H), 7.94-7.86 (m, 3H), 7.78 (d, J = 1.5 Hz, 1H), 7.31 (d, J = 8.4, 1H), 4.40 (m, 1H), 3.83 (s, 3H), 3.79 (t, J = 6.6, 2H), 3.72 (d, J = 7.1, 2H), 3.05 (m, 2H), 2.97 (m, 2H), 2.60 (m, 2H), 2.05 (d, J = 12.5, 2H), 1.89-1.79 (m, 4H).

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EXAMPLE 36

1,1-Dioxido-2-(4-(4-(5-ethylsulfonyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

5

From the hydrochloride salt of 4-(5-ethylsulfonyl-2-oxo-3-benzoxazoliny)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.01 (t, J = 6.0 Hz, 2H), 7.93-7.87 (m, 2H), 7.77 (s, 1H), 7.67 (d, J = 7.1 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 4.48 (m, 1H), 3.79 (m, 2H), 3.60 (d, J = 11.2 Hz, 2H), 3.14-3.06 (overlapping q & m, 6H), 2.61 (br q, J = 12.5 Hz, 2H), 2.10 (d, J = 13.4 Hz, 2H), 1.85 (m, 4H), 1.15 (t, J = 6.2 Hz, 3H),

15

EXAMPLE 37

1,1-Dioxido-2-(4-(4-(2-oxo-3-oxazolo[4,5-b]pyridyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

20

From the hydrochloride salt of 4-(2-oxo-3-oxazolo[4,5-b]pyridyl)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.03-7.99 (m, 3H), 7.94-7.85 (2 overlapping td, 2H), 7.49 (dd, J = 8.0, 1.2 Hz, 1H), 7.07 (dd, J = 8.0, 5.0 Hz, 1H), 4.56 (tt, J = 12.1, 4.1 Hz, 1H), 3.79 (t, J = 6.4 Hz, 2H), 3.63 (t, J = 12.8 Hz, 2H), 3.20-3.09 (m, 4H), 2.82 (qd, J = 13.3, 3.5 Hz, 2H), 2.09 (d, J = 13.4, 2H), 1.88-1.78 (m, 4H).

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EXAMPLE 38**1,1-Dioxido-2-(4-(4-(7-carbethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

5 From the hydrochloride salt of 4-(7-carbethoxy-2-oxo-3-benzoxazoliny)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid, HCl salt: ¹H NMR (400 MHz, CD₃OD) 8.01 (td, J = 6.4, 1.5 Hz, 2H), 7.94-7.86 (m, 2H), 7.63
10 (dd, J = 8.1, 1.1 Hz, 1H), 7.47 (dd, J = 7.9, 1.1 Hz, 1H), 7.26 (t, J = 8.1 Hz, 1H), 4.42 (tt, J = 12.1, 4.0 Hz, 1H), 3.87 (s, 3H), 3.80 (t, J = 6.2, 2H), 3.63 (d, J = 12.8 Hz, 2H), 3.16-3.06 (m, 4H), 2.65 (qd, J = 13.0, 4.0 Hz, 2H), 2.09 (d, J = 13.5 Hz, 2H), 1.89-1.83 (m, 4H).

15

EXAMPLE 39**1,1-Dioxido-2-(4-(4-(5-tert-butyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

20 From the hydrochloride salt of 4-(5-tert-butyl-2-oxo-3-benzoxazoliny)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃) 8.07 (dd, J = 6.6, 2.25 Hz, 1H), 7.88 (m, 3H), 7.19 (s, 1H),
25 7.11 (s, 2H), 4.17 (tt, J = 12.21, 3.66 Hz, 1H), 3.85 (t, J = 7.14 Hz, 2H), 3.09 (d, J = 10.85 Hz, 2H), 2.46 (t, J = 7.38 Hz, 2H), 2.36 (dt, J = 12.15, 3.5 Hz, 2H), 2.13 (dt, J = 10.89, 1.27 Hz, 2H), 1.91 (m, 4H), 1.66 (m, 2H), 1.342 (s, 9H).

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EXAMPLE 40**1,1-Dioxido-2-(4-(4-(5,7-dimethyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

5 From the hydrochloride salt of 4-(5,7-dimethyl-2-oxo-3-benzoxazoliny)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃) 8.08 (d, J = 7.27 Hz, 1H), 7.90 (m, 3H), 7.65 (br m, 1H),
10 6.752 (s, 1H), 4.55 (br m, 1H), 3.85 (br t, 2H), 3.70 (br m, 2H), 3.32 (br m, 2H), 3.10 (br m, 2H), 2.87 (br m, 2H), 2.413 (s, 3H), 2.318 (s, 3H), 2.06 (br m, 6H).

EXAMPLE 41**2-(4-Bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one**

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, 21, 583-4 was modified: A mixture of 6 g of sodium saccharin, 100 mL of 1,4-dibromobutane, and 5 mL of N,N-dimethylformamide was heated at 50°C overnight. After cooling to
20 ambient temperature, the mixture was diluted with 250 mL of ether and 50 mL of water. The aqueous layer was extracted with two additional 50 mL portions of ether and the combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure.
25 The excess 1,4-dibromobutane was removed by short path vacuum distillation and the oily residue purified by crystallization from ether-hexane, mp 71-2°C.

EXAMPLE 42**2-(4-Bromobutyl)-1,1-dioxido-5-methoxy-1,2-benzothiazol-3(2H)-one**

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, 21, 583-4 was modified: A mixture of 0.5 g of

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3,3-dioxido-5-methoxy-1,2-benzothiazol-3(2H)-one prepared as described by J. G. Lombardino, *J. Org. Chem.* **1971**, 36, 1843-5, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave 0.8 g of crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

EXAMPLE 43

2-(4-Bromobutyl)-1,1-dioxido-5-nitro-1,2-benzothiazol-3(2H)-one

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, 21, 583-4 was modified: A stirred mixture of 1.0 g of 3,3-dioxido-5-nitro-1,2-benzothiazol-3(2H)-one prepared as described by W. S. Saari and J. E. Schwering, *J. Heterocyclic Chem.* **1986**, 23, 1253, and 6 mL of 1N NaOH was warmed to dissolution and allowed to cool to ambient temperature for 15 min. The mixture was concentrated to dryness under reduced pressure and the white solid azeotropically dried with 20 mL of toluene. The resulting sodium salt was dissolved in 3 mL of N,N-dimethylformamide and 2.6 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave 1.1 g of crude product as a solid which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification: ¹H NMR (300 MHz, CDCl₃): 8.87 (d, J = 0.5 Hz, 1H), 8.74 (dd, J = 8.38, 2.03 Hz, 1H), 8.15 (dd, J = 8.41, 2.0 Hz, 1H), 3.88 (t, J = 6.8 Hz, 2H), 3.47 (t, J = 6.3 Hz, 2H), 2.02 (m, 4H).

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EXAMPLE 44**1,1-Dioxido-2-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one**

- 5 Step 1: To a ice cold, stirred mixture of 55 g of ethyl isonipecotate, 200 mL of ether, 200 mL of saturated sodium carbonate and 200 mL of water was added dropwise 40 mL of benzoyl chloride. The resulting mixture was allowed to warm and stir overnight, diluted with 200 mL of ethyl acetate and separated. The organic layer was washed with 200
10 mL of 1N hydrochloric acid, dried over MgSO₄, and concentrated. Drying the residue under vacuum gave 83.3 g (92%) of ethyl 1-benzoyl-4-piperidinecarboxylate as a white solid.
- 15 Step 2: A stirred solution of 176 mL of commercial 1M lithium bistrimethylsilylamide in tetrahydrofuran was diluted with 100 mL of tetrahydrofuran and cooled to -78°C under inert atmosphere. A solution of 44 g of ethyl 1-benzoyl-4-piperidinecarboxylate in 200 mL of tetrahydrofuran was added dropwise keeping the temperature below -60°C, followed by 21 mL of benzyl bromide. The resulting mixture
20 was allowed to warm and stir overnight, diluted with 200 mL of 1N hydrochloric acid, 200 mL of ethyl acetate and separated. The organic layer was washed with 200 mL of 1N hydrochloric acid, dried over MgSO₄, and concentrated. The residue was dissolved in 300 mL of toluene, filtered and concentrated under reduced pressure. Drying
25 under reduced pressure gave 56.3 g of ethyl 1-benzoyl-4-(phenylmethyl)-4-piperidinecarboxylate as a viscous oil homogeneous by thin layer chromatography, eluting with 1:1 ethyl acetate: hexane, ¹H NMR (300 MHz, CDCl₃) 1.20 (t, 3H), 1.40 (m, 1H), 1.60 (m, 1H), 2.07 (m, 1H), 2.22 (m, 1H), 2.85 (d, 2H), 3.08 (m, 1H), 3.65 (m, 1H), 4.12 (q, 2H), 4.65 (m, 1H), 7.04 (m, 2H), 7.24 (m, 3H), 7.38 (m, 5H).
30 Step 2: A mixture of 65 g of ethyl 1-benzoyl-4-(phenylmethyl)-4-piperidinecarboxylate, 300 g of polyphosphoric acid and 500 mL of xylene was heated at reflux overnight. The xylene layer was decanted off and the residue washed with two additional 100 mL portions of xylene. The residue was dissolved in 1L of water and 1L of ethyl

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acetate and separated. The aqueous was extracted with two additional 200 mL portions of ethyl acetate and the combined organic extracts were washed with 500 mL of sat'd Na₂CO₃, water and dried over MgSO₄. Removal of solvents under reduced pressure gave 27 g of 1'-benzoyl-1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) as crystalline solid, mp 149-154°C; ¹H NMR (300 MHz, CDCl₃) 1.50 (m, 2H), 2.00 (m, 2H), 3.12 (m, 2H), 3.25 (ddd, 2H), 3.90 (m, 1H), 4.62 (m, 1H), 7.43 (m, 1H), 7.63 (dd, 1H), 7.79 (d, 1H).

Step 3: A mixture of 27 g of 1'-benzoyl-1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) 400 mL of ethanol and 100 mL of conc. hydrochloric acid was heated at reflux for 48 hrs. The mixture was concentrated to remove ethanol, diluted 200 mL with water and extracted with two additional portions of ethyl acetate. The aqueous layer was basified with 20% NaOH and extracted with three 200 mL portions of ethyl acetate. The combined organic extracts were washed with 50 mL of water and dried over MgSO₄. Removal of solvents under reduced pressure gave 18 g of 1,3-dihydro-1-oxo-spiro(2H-indene-2,4'-piperidine) as brown solid product: ¹H NMR (300 MHz, CDCl₃) 1.35 (dd, 2H), 1.62 (br s, 1H), 1.88 (ddd, 2H), 2.81 (ddd, 2H), 3.10 (s, 2H), 3.17 (m, 2H), 7.38 (t, 1H), 7.46 (d, 1H), 7.60 (t, 1H), 7.77 (d, 1H).

Step 4: From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine), and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained 1,1-dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one as a white solid: ¹H NMR (400 MHz, CDCl₃): 8.06 (d, J=6.8 Hz, 1H), 7.92 (d, J=6.6 Hz, 1H), 7.89-7.83 (m, 2H), 7.75 (d, J=7.7 Hz, 1H), 7.59 (t, J=7.4 Hz, 1H), 7.44 (d, J=6.96 Hz, 1H), 7.37 (t, J=7.4 Hz, 1H), 3.83 (t, J=7.4 Hz, 2H), 3.02 (s, 2H), 2.95 (t, J=11.36 Hz, 2H), 2.45 (t, J=7.4 Hz, 2H), 2.17-2.01 (m, 4H), 1.94-1.90 (m, 2H), 1.67-1.64 (m, 2H), 1.38 (br d, J=12 Hz, 2H).

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EXAMPLE 45

1,1-Dioxido-2-(4-(4'-(3,4-dihydro-1-oxonaphthalene)-2(1H)-spiropiperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one

5 From 4'-(3,4-dihydro-1-oxonaphthalene)-2(1H)-spiropiperidine, prepared as described by P. J. Gilligan, et al., *J. Med. Chem.* **1994**, 37, 364-370, and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was
10 obtained a white solid: mp 226-8°C.

EXAMPLE 46

4-(3,4-Dihydro-6-methyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide

15 From 3,4-dihydro-6-methyl-spiro[2H-1-benzopyran-2,4'-piperidine, and 2-(4-bromobutyl)-phthalimide using the procedure described for Example 15, Step 5 there was obtained a white solid: mp >275°C dec.

EXAMPLE 47

20 **4-(Spiro(piperidine-4,6'-[6H]thieno[2,3-b]thiopyran-4'(5'H)-one-1'-yl)-butylphthalimide**

From 4-(spiro(piperidine-4,6'-[6H]thieno[2,3-b]thiopyran-4'(5'H)-one, and 2-(4-bromobutyl)-phthalimide using the procedure described for
25 Example 15, Step 5 there was obtained a white solid: mp 261-3°C dec.

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EXAMPLE 48**4-(Spiro[benzothiazol-2(3H),4'-piperidin-1'-yl]-butylphthalimide**

- Step 1: A solution of 4-piperidone hydrochloride hydrate (2 gram, 13 mmol), bromobutylphthalimide (3.67 gram, 13 mmol) and triethylamine (3.4 ml, 26 mmol) in Chloroform (25 ml) was refluxed overnight. The reaction mixture was washed with sat. NaHCO₃ and was dried over Na₂SO₄. Purification using flash chromatography gave 4-(4'-ketopiperidiny)butylphthalimide (0.85 gram).
- Step 2: A solution of 4-(4'-ketopiperidiny)butylphthalimide (200 mg, 0.67 mmol), 2-aminothiophenol (125ml, 0.73mmol) and tosic acid (30 mg) in benzene (25 ml) was refluxed overnight. The reaction mixture was concentrated and the residue was partitioned between ethyl acetate and sat. NaHCO₃. The organic layer was dried over Na₂SO₄.
- Chromatography gave a white solid: ¹H NMR (300 MHz, CDCl₃): 8.01-7.95 (m, 2H), 7.88-7.82 (m, 2H), 7.17 (d, J=7.57 Hz, 1H), 7.04 (t, 1H), 6.86 (t, J=3.76 Hz, 1H), 6.78 (d, J=7.63 Hz, 1H), 4.14 (br s, 1H), 3.85 (t, J=7.04 Hz, 2H), 2.89-2.06 (m, 2H), 2.54-2.30 (m, 6H), 2.18-2.06 (m, 2H), 1.90-1.72 (m, 2H), 1.69-1.37 (m, 2H).

EXAMPLE 49**4-(3,4-Dihydro-6-methoxy-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide**

- From 3,4-dihydro-6-methoxy-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one and 2-(4-bromobutyl)-phthalimide using the procedure described for Example 15, Step 5 there was obtained a white solid:mp 11-113°C.

EXAMPLE 50**4-(3,4-Dihydro-6-methanesulfonylamidyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide**

From 3,4-dihydro-6-methanesulfonylamidyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one and 2-(4-bromobutyl)-phthalimide using the

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procedure described for Example 15, Step 5 there was obtained a white solid: mp 89-90°C.

EXAMPLE 51

5 **1,1-Dioxido-2-(4-(spiro[benzothiazol-2(3H),4'-piperidin-1'-yl)-butyl]-1,2-benzisothiazol-3(2H)-one**

From 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one, 4-piperidone and 2-aminothiophenol using the procedure described for Example 48 there was obtained a white solid: mp 178-80°C.

EXAMPLE 52

4-(6-Trifluoromethyl-spiro[benzothiazol-2(3H),4'-piperidin-1'-yl]-butylphthalimide

15 From 4-(4'-ketopiperidiny)butylphthalimide and 2-amino-5-trifluoromethylthiophenol using the procedure described for Example 48, Step 2 there was obtained a white solid: mp 157-8°C.

EXAMPLE 53

20 **1,1-Dioxido-2-(4-(spiro[benzofuran-2(3H),4'-piperidin]-1'-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

From spiro[benzofuran-2(3H),4'-piperidine] prepared as described by R. C. Effland, et al. *J. Heterocyclic Chem.* **1981**, 18, 811, and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 98-100°C.

EXAMPLE 54

30 **4-(Spiro[benzofuran-2(3H),4'-piperidin]-1'-yl)-butylphthalimide**

From spiro[benzofuran-2(3H),4'-piperidine] prepared as described by R. C. Effland, et al. *J. Heterocyclic Chem.* **1981**, 18, 811, and 2-(4-bromobutyl)-phthalimide using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 255-7°C.

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EXAMPLE 55**4-(Spiro[2H-1,3-benzoxazine-2,4'-piperidin]-1'-yl)-butylphthalimide**

5 From spiro[2H-1,3-benzoxazine-2,4'-piperidine] and 2-(4-bromobutyl)-phthalimide using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 165-70°C.

EXAMPLE 56

10 **3,3-Dioxido-1,2-dehydro-2-(4-(spiro[2H-indenyl-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-naphth[1,2-d]isothiazol-1-one**

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-bromobutyl)-3,3-dioxido-1,2-dehydronaphth[1,2-d]isothiazol-1-one
15 using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃): 9.27 (d, J=8.4 Hz, 1H), 8.30 (d, J=8.5 Hz, 1H), 8.00 (d, J=7.6 Hz, 1H), 7.88 (d, J=8.5 Hz, 1H), 7.82-7.73 (m, 3H), 7.56 (t, 1H), 7.43 (d, J=7.6 Hz, 1H), 7.36 (t, 3H), 3.85 (t, J=7.4 Hz, 2H), 3.01 (s, 2H), 2.97 (br d, 2H), 2.14-2.0 (m, 4H), 1.90 (m, 8H), 1.69 (m, 2H), 1.38 (br d, J=12 Hz, 2H); mp (HCl salt) 243-50°C.

EXAMPLE 57

25 **1,1-Dioxido-2-(4-(spiro[2H-indenyl-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one**

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one
30 using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃): 7.83-7.74 (m, 2H), 7.61 (t, J=7.4 Hz, 1H), 7.56 (m, 2H), 7.34 (t, J=7.7 Hz, 1H), 7.29 (m, 1H), 4.05 (s, 3H), 3.78 (t, J=7.3 Hz, 2H), 3.02 (s, 2H), 2.96 (br d, J=11 Hz, 2H), 2.43 (t, J=7.6 Hz, 2H), 2.17-2.0 (m, 4H), 1.87 (m, 2H), 1.65 (m, 2H), 1.31 (br d, J=12 Hz, 2H).

EXAMPLE 58

1,1-Dioxido-2-(4-(spiro[2H-indenyl-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-7-methoxy-1,2-benzisothiazol-3(2H)-one

- 5 From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-7-methoxy-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃): 7.77-7.72 (m, 2H), 7.61-56 (m, 2H), 7.44 (d, J=7.6 Hz, 1H), 7.37 (t, 1H), 7.28 (d, 1H), 4.05 (s, 3H), 3.80 (t, J=7.3 Hz, 2H), 3.02 (s, 2H), 2.96 (m, 2H), 2.44 (t, J=7.5 Hz, 2H), 2.13-2.03 (m, 4H), 1.89 (m, 2H), 1.65 (m, 2H), 1.39 (br d, J=12 Hz, 2H); mp (HCl salt) 130°C (dec).

EXAMPLE 59

- 15 **1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-5-methoxy-1,2-benzisothiazol-3(2H)-one**

- 20 From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-5-methoxy-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃): 7.80 (d, J=8.6 Hz, 1H), 7.75 (d, J=7.6 Hz, 1H), 7.6 (d, J=7.6 Hz, 1H), 7.59 (t, 1H), 7.48-7.37 (m, 2H), 7.32 (t, J=7.8 Hz, 1H), 7.29 (d, 1H), 3.95 (s, 3H), 3.81 (t, J=7.4 Hz, 2H), 3.02 (s, 2H), 2.95 (br d, J=11 Hz, 2H), 2.44 (t, J=7.4 Hz, 2H), 2.14-2.0 (m, 4H), 1.90 (m, 2H), 1.65 (m, 2H), 1.38 (br d, J=12 Hz, 2H); mp (HCl salt) 205-7°C.

EXAMPLE 60

- 30 **1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-6-methoxy-1,2-benzisothiazol-3(2H)-one**

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-6-methoxy-1,2-benzisothiazol-3(2H)-one

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using the procedure described for Example 15, Step 5 there was obtained a white solid:rom spiro[2H-indene-2,4'-piperidine and 2-(4-bromobutyl)-5-methoxy-1,2-benzisothiazol-3(2H)-one using the
5 procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃): 7.94 (d, J=8.6 Hz, 1H), 7.75 (d, J=7.6 Hz, 1H), 7.6 (t, 1H), 7.44 (d, J=7.6 Hz, 1H), 7.39-7.34 (m, 2H), 7.27-7.25 (m, 1H), 3.97 (s, 3H), 3.79 (t, J=7.3 Hz, 2H), 3.02 (s, 2H), 2.96 (m, 2H), 2.44 (br t, 2H), 2.14-2.0 (m, 4H), 1.89 (m, 2H), 1.65 (m, 2H), 1.38 (br d, 2H); mp (HCl salt) 229-31°C.
10

EXAMPLE 61

1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-5-methyl-1,2-benzisothiazol-3(2H)-one

15 From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-5-methyl-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃): 7.86-7.76 (m, 3H), 7.68-7.58 (m, 2H), 7.46 (d, J= 6.8 Hz, 1H), 7.39 (t, J=7.4 Hz, 1H), 3.83 (t, J=6.8
20 Hz, 2H), 3.03 (s, 2H), 2.98 (br d, J=10.6 Hz, 2H), 2.57 (s, 3H), 2.46 (t, J=7.4 Hz, 2H), 2.19-2.05 (m, 4H), 1.92 (m, 2H), 1.66 (m, 2H), 1.40 (br d, 11.6 Hz, 2H); mp (HCl salt) 230-1°C.

EXAMPLE 62

25 **1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one**

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-5-chloro-1,2-benzisothiazol-3(2H)-one using
30 the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃): 8.02 (s, 1H), 7.87-7.80 (m, 2H), 7.75 (d, J=7.6 Hz, 1H), 7.59 (t, J=7.6 Hz, 1H), 7.44 (d, J=7 Hz, 1H), 7.37 (t, J=7.5 Hz, 1H), 3.82 (t, J=7.4 Hz, 2H), 3.02 (s, 2H), 2.95

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(br d, J=11.5 Hz, 2H), 2.44 (t, 2H), 2.16-2.0 (m, 4H), 1.90 (m, 2H), 1.64 (m, 2H), 1.38 (br d, J=11.5 Hz, 2H); mp (HCl salt) 235-7°C.

EXAMPLE 63

5 **1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one**

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one using
10 the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃): 7.93 (m, 1H), 7.76-7.71 (m, 2H), 7.61-7.45 (m, 2H), 7.44 (d, J=7.7 Hz, 1H), 7.37 (t, J=7.4 Hz, 1H), 3.82 (t, J=7.4 Hz, 2H), 3.02 (s, 2H), 2.97 (br d, J=11.5 Hz, 2H), 2.45 (t, J=7.4 Hz, 2H), 2.16 (m, 2H), 2.05 (m, 2H), 1.95-1.87 (m, 2H), 1.7-1.6 (m, 2H), 1.38 (br d, J=12.5 Hz, 2H); mp (HCl salt) 238-40°C.

EXAMPLE 64

20 **1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one**

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-5-nitro-1,2-benzisothiazol-3(2H)-one using
25 the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃): 8.84 (s, 1H), 8.70 (d, J=6.38 Hz, 1H), 8.11 (d, J=8.4 Hz, 1H), 7.73 (d, J=7.6 Hz, 1H), 7.56 (d, J=8.4 Hz, 1H), 7.43 (d, J=7.7 Hz, 1H), 7.35 (t, 1H), 3.86 (t, J=7.4 Hz, 2H), 3.00 (s, 2H), 2.95 (br d, 2H), 2.42 (t, J=7.4 Hz, 2H), 2.14 (m, 2H), 2.04 (m, 2H), 1.94 (m, 2H), 1.63 (m, 2H), 1.37 (br d, J=12.5 Hz, 2H);
30 mp (HCl salt) 241-3°C.

EXAMPLE 65**1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-6-nitro-1,2-benzisothiazol-3(2H)-one**

5 From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1,1-dioxido-2-(4-bromobutyl)-6-nitro-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃): 8.75 (s, 1H), 8.68 (dd, J= 2, 6.4 Hz, 1H), 8.26 (d, J=8.3 Hz, 1H), 7.75 (d, J= 7.5 Hz, 1H), 7.59 (t, 1H), 4.25 (br d, J=12.5 Hz, 2H), 3.88 (t, J=7.4 Hz, 2H), 3.02 (s, 2H), 2.96 (br d, 2H), 2.44 (t, J=7.4 Hz, 2H), 2.13-1.91 (m, 6H), 1.63 (m, 2H); mp (HCl salt) 231-4°C.

EXAMPLE 66

15 **1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one**

Step 1: A solution of 16 g of pyridine-3-sulfonyl chloride prepared as described by B. I. Alo, O. B. Familoni, F. Marsais and G. Queguiner, 20 *J. Heterocyclic Chem.* **1992**, 29, 61-4 in 250 mL of chloroform was added to a solution of 8 g of 4-amino-1-butanol and 40 mL of triethylamine in 250 mL of chloroform at 0°C. The mixture was stirred overnight while warming to ambient temperature, the concentrated under reduced pressure to dryness. The residue was 25 stirred with 500 mL of ethyl acetate, filtered and concentrated to dryness. To a solution of the crude N-(4-hydroxybutyl)pyridine-3-sulfonamide (28 g) in 200 mL of dichloromethane was added 30 mL of 3,4-dihydro-2H-pyran and 3 g of p-toluenesulfonic acid monohydrate. The reaction was stirred overnight, shaken with 100 mL of saturated sodium bicarbonate, dried over MgSO₄, and concentrated to dryness. Chromatography of the residue over silica gel, eluting with ethyl acetate gave 20 g of N-(4-(2-tetrahydropyranyloxy)butyl)pyridine-3-sulfonamide as a thick amber oil.

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Step 2: To a stirred solution of 54 mL of dry diisopropylamine in 200 mL of dry tetrahydrofuran cooled to -78°C was added 205 mL of commercial 1.6M n-butyllithium in hexane keeping the temperature below -30°C . After aging for 30 min -30°C , the solution was cooled to -78°C a solution of 23.3 g of N-(4-(2-tetrahydropyranyloxy)butyl)pyridine-3-sulfonamide in 150 mL of dry tetrahydrofuran was added dropwise keeping the internal temperature below -70°C (ca. 30 min). The resulting solution was aged at -78°C for 2.5 h and the deep red solution quenched by a stream of carbon dioxide gas with a fritted glass dispersion tube. During the addition, the color dissipated and the temperature rose to -40°C . After 15 min below -40°C , the bath was removed and the solution allowed to warm to room temperature over 1 h under a gentle stream of carbon dioxide. The mixture was partitioned between 400 mL of water and 500 mL of ether. The ether extracts contained unreacted N-(4-(2-tetrahydropyranyloxy)butyl)pyridine-3-sulfonamide. The aqueous layer was carefully acidified to $\text{pH}=3$ with concentrated HCl, and extracted with five 200 mL portions of chloroform. The combined organic extracts were dried over MgSO_4 and concentrated under reduced pressure. Drying under vacuum gave 19 g of N-(4-(2-tetrahydropyranyloxy)butyl) 4-carboxypyridine-3-sulfonamide as a amber solid.

Step 3: To a stirred solution of 19 g of N-(4-(2-tetrahydropyranyloxy)butyl) 4-carboxypyridine-3-sulfonamide and 16 mL of triethylamine in 300 mL of dichloromethane at 0°C was added 6.5 mL of methyl chloroformate dropwise. After 30 min, the mixture was shaken with 100 mL of saturated sodium bicarbonate, dried over MgSO_4 and concentrated under reduced pressure. Chromatography of the dark brown oil over silica gel, eluting with 1:1 ethyl acetate: hexane gave 16 g of homogeneous 1,1-dioxido-2-(4-(2-tetrahydropyranyloxy) butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one as an amber oil.

Step 4: A solution of 1 g of 2-(4-(2-tetrahydropyranyloxy)butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one and 0.1 g of p-toluenesulfonic acid monohydrate in 50 mL of methanol was stirred overnight at ambient

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temperature and concentrated to dryness under reduced pressure. The residue was dissolved in 200 mL of ethyl acetate, washed with 4 mL of saturated sodium bicarbonate, dried over MgSO₄ and concentrated under reduced pressure. Drying under vacuum overnight gave 0.73 g (78%) of 2-(4-hydroxybutyl)-isothiazolo[5,4-c]pyridin-3(2H)-one as an oil: ¹H NMR (400 MHz, CDCl₃): 9.28 (s, 1H), 9.15 (d, J=5 Hz, 1H), 7.98 (d, J=5 Hz, 1H), 3.86 (t, J= 7.5 Hz, 2H), 3.71 (t, J= 6.4 Hz, 2H), 2.36 (br s, 1H), 1.98 (m, 2H), 1.27 (m, 2H).

Step 4: To a stirred solution of 0.73 g of 2-(4-hydroxybutyl)-isothiazolo[5,4-c]pyridin-3(2H)-one and 0.8 mL of triethylamine in 50 mL of dry tetrahydrofuran at 0°C was added 0.63 g of technical grade 4-nitrobenzenesulfonyl chloride. After 4 h, the solution was filtered and concentrated to dryness under reduced pressure. The residue was dissolved in 100 mL of ethyl acetate, washed with 10 mL of saturated sodium carbonate and dried over MgSO₄. Concentration under reduced pressure gave 1.4 g of crude 2-(4-(4-nitrobenzenesulfonyloxy)butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one as an oil. The crude product was a mixture of 4-nitrobenzenesulfonate contaminated with a small amount of chloride by NMR, and was used in the next step without further purification.

Step 6: A mixture of 0.60 g of 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1.4 g of 1,1-dioxido-2-(4-(4-nitrobenzenesulfonyloxy)butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one in 20 mL of anhydrous acetonitrile were allowed to stir overnight at ambient temperature then concentrated to dryness under reduced pressure. The solid mass was partitioned between 200 mL of ethyl acetate and 20 mL of saturated sodium carbonate. The organic extract was dried over MgSO₄ and concentrated under reduced pressure.

Chromatography of the residue on silica gel, eluting with 10% methanol in ethyl acetate gave 0.5 g of a white solid: ¹H NMR (400 MHz, CDCl₃): 9.26 (s, 1H), 9.15 (m, 1H), 7.95 (d, J=4.9 Hz, 1H), 7.75 (d, J= 7.5 Hz, 1H), 7.59 (t, J=8.1 Hz, 1H), 7.44 (d, J=7.7 Hz, 1H), 3.85 (t, J=7.4 Hz, 2H), 3.02 (s, 2H), 2.95 (br d, J=12 Hz, 2H), 2.44 (t, J=7.4

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Hz, 2H), 2.17-2.03 (m, 4H), 1.92 (m, 2H), 1.64 (m, 2H), 1.4 (br d, J=12 Hz, 2H); mp (HCl salt) 228-30°C.

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EXAMPLE 67

1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one

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Step 1: Chlorine gas was dispersed through a mixture of 4.3 g of methyl 2-mercaptopyridine-3-carboxylate, prepared as described by L. Katz, W. Schroeder and M. Cohen, *J. Org. Chem.* **1954**, *19*, 711-17, 10 mL of dichloromethane, 60 mL of glacial acetic acid and 150 mL of water at 0-3°C for 1.5 h. The resulting solution was extracted with four 200 mL portions of chloroform and the combined extracts dried over MgSO₄ and concentrated under reduced pressure in a fume hood. The oily methyl 2-chlorosulfonylpyridine-3-carboxylate, 3.5 g, was dissolved in 20 mL of dichloromethane and added to a solution of 2 g of 4-amino-1-butanol and 3 mL of triethylamine in 200 mL of dichloromethane at 0°C. The mixture was stirred overnight while warming to ambient temperature, the decanted and the residue extracted with three 100 mL portions of dichloromethane. The combined extracts were concentrated under reduced pressure to dryness. To a solution of the resulting crude N-(4-hydroxybutyl) 3-methoxycarbonylpyridine-2-sulfonamide (5 g) in 100 mL of dichloromethane was added 10 mL of 3,4-dihydro-2H-pyran and 1 g of p-toluenesulfonic acid monohydrate. The reaction was stirred overnight, shaken with 50 mL of saturated sodium bicarbonate, dried over MgSO₄, and concentrated to dryness. To a stirred solution of the resulting crude N-(4-(2-tetrahydropyranyloxy)butyl) 3-methoxycarbonylpyridine-2-sulfonamide (7 g) in 100 mL of 100 mL of methanol and 30 mL of water was added 2 g of lithium hydroxide monohydrate. The reaction mixture was stirred overnight at ambient temperature, concentrated to dryness under reduced pressure to remove methanol, acidified to pH=3 with concentrated HCl and extracted with five 50 mL portions

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of chloroform. The combined extracts were dried over MgSO_4 and concentrated to dryness. The residue, N-(4-(2-tetrahydropyranyloxy)butyl) 3-carboxycarbonylpyridine-2-sulfonamide weighed 5 g and was used without further purification.

5 Step 2: To a stirred solution of 3 g of N-(4-(2-tetrahydropyranyloxy)butyl) 3-carboxycarbonylpyridine-2-sulfonamide and 2.3 mL of triethylamine in 100 mL of dichloromethane at 0°C was added 0.8 mL of methyl chloroformate dropwise. After 2 h, the mixture was shaken with 10 mL of saturated sodium bicarbonate, dried over MgSO_4 and concentrated under reduced pressure. Chromatography of the dark brown oil over silica gel, eluting with 1:1 ethyl acetate: hexane gave 3 g of homogeneous 1,1-dioxido-2-(4-(2-tetrahydropyranyloxy)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one as an amber oil.

15 Step 3: A solution of 1 g of 2-(4-(2-tetrahydropyranyloxy)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one and 0.1 g of p-toluenesulfonic acid monohydrate in 50 mL of methanol was stirred overnight at ambient temperature and concentrated to dryness under reduced pressure. The residue was dissolved in 200 mL of ethyl acetate, washed with 4 mL of saturated sodium bicarbonate, dried over MgSO_4 and concentrated under reduced pressure. Drying under vacuum overnight gave 0.73 g of 1,1-dioxido-2-(4-hydroxybutyl)-isothiazolo[5,4-b]pyridin-3(2H)-one as an oil: ^1H NMR (400 MHz, CDCl_3): 9.00 (d, $J=1.5$ Hz, 1H), 8.40 (d, $J=7.7$ Hz, 1H), 7.80 (dd, $J=1.5, 7.7$ Hz, 1H), 3.86 (t, $J=7.5$ Hz, 2H), 3.71 (t, $J=6.4$ Hz, 2H), 2.31 (br s, 1H), 1.98 (m, 2H), 1.26 (m, 2H).

25 Step 4: To a stirred solution of 0.73 g of 1,1-dioxido-2-(4-hydroxybutyl)-isothiazolo[5,4-b]pyridin-3(2H)-one and 0.8 mL of triethylamine in 50 mL of dry tetrahydrofuran at 0°C was added 0.63 g of technical grade 4-nitrobenzenesulfonyl chloride. After 4 h, the solution was filtered and concentrated to dryness under reduced pressure. The residue was dissolved in 100 mL of ethyl acetate, washed with 10 mL of saturated sodium carbonate and dried over MgSO_4 . Concentration under reduced pressure gave 1.4 g of crude

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1,1-dioxido-2-(4-(4-nitrobenzenesulfonyloxy)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one as an oil. The crude product was a mixture of 4-nitrobenzenesulfonate contaminated with a small amount of chloride by NMR, and was used in the next step without further purification.

5 Step 5: A mixture of 0.60 g of 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 1.4 g of 1,1-dioxido-2-(4-(4-nitrobenzenesulfonyloxy)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one in 20 mL of anhydrous acetonitrile were allowed to stir overnight at ambient temperature then concentrated to dryness under reduced

10 pressure. The solid mass was partitioned between 200 mL of ethyl acetate and 20 mL of saturated sodium carbonate. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with 10% methanol in ethyl acetate gave 0.5 g of a white solid: ¹H NMR (400 MHz, CDCl₃): 9.00(d, J=4.8 Hz, 1H), 8.38 (d, J=7.7 Hz, 1H), 7.81-7.74 (m, 2H), 7.59 (t, J= 7.4 Hz, 1H), 7.45 (d, J=7 Hz, 1H), 7.37 (t, J=7.4 Hz, 1H), 3.87 (t, J=7.23 Hz, 1H), 3.02 (s, 2H), 2.97 (br d, J=11.35 Hz, 2H), 2.46 (t, J=7.33 Hz, 2H), 2.20-2.03 (m, 4H), 2.07-2.20 (m, 2H), 1.97-1.90 (m, 2H), 1.70-1.61 (m, 2H), 1.61 (br d, J=12.08 Hz, 2H).

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EXAMPLE 68

1,1-Dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one

25 From 4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidine and 2-(4-bromobutyl)-1,1-dioxido-5-nitro-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR, HCl salt, (400 MHz, CDCl₃): 8.87 (d, J = 2.0 Hz, 1H), 8.74 (d, J = 2.18 Hz, 1H), 8.72 (d, J = 1.8 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 6.54 Hz, 1H), 7.03 (s, 1H), 6.94 (d, J = 8.06 Hz, 1H),

30 4.18 (m, 1H), 3.89 (t, J = 7.39 Hz, 2H), 3.06 (d, J = 10.07 Hz, 2H), 2.46 (m, 2H), 2.38 (s, 3H), 2.34 (m, 2H), 2.14 (t, J = 11.5 Hz, 2H), 1.94 (m, 2H), 1.85 (d, J = 11.4 Hz, 2H), 1.65 (m, 2H).

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EXAMPLE 69

2-(4-(Spiro(1,3-dihydro-1-oxo-2H-indenyl-2,4'-piperidin-1'-yl)butyl)-pyrrolo[3,4b]pyridin-5,7(1H)-dione

- 5 From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-bromobutyl)-pyrrolo[3,4b]pyridin-5,7(1H)-dione using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR, (400 MHz, CDCl₃): 9.15 (s, 1H), 9.06 (s, 1H), 9.06 (d, J=4.76 Hz, 1H), 7.75 (d, J=4.76 Hz, 1H), 7.59 (t, J=7.42 Hz, 1H), 7.44 (d, J=8.0Hz, 1H), 7.37 (t, J=7.33 Hz, 1H), 3.76 (t, J=7.14 Hz, 2H), 3.02 (s, 2H), 2.41 (br t, J=7.33 Hz, 2H), 2.12-1.99 (m, 4H), 1.77-1.72 (m, 2H), 1.56 (br m, 2H), 1.38 (m, 2H).

EXAMPLE 70

- 15 **1,1-Dioxido-2,3-dihydro-2-(4-(spiro[2H-indenyl-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-naphth[1,8-de]isothiazin-3-one**

- From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-bromobutyl)-1,1-dioxido-2,3-dihydronaphth[1,8-de]isothiazin-3-one using the procedure described for Example 15, Step 5 there was obtained a white solid: ¹H NMR (300 MHz, CDCl₃): 8.37 (d, J=7.92 Hz, 1H), 8.26 (d, 1H), 8.08 (m, 2H), 7.86-7.74 (m, 3H), 7.58 (t, 1H), 7.44 (d, J=7.7 Hz, 1H), 7.38 (t, 1H), 3.90 (t, J=7.3 Hz, 2H), 3.02 (s, 2H), 2.98 (br d, 2H), 2.46 (t, J=7.4 Hz, 2H), 2.14-1.94 (m, 6H), 1.69 (m, 2H), 1.40 (br d, 2H); mp (HCl salt) 248-50°C.

EXAMPLE 71

2-(4-Bromobutyl)-1,1-dioxido-4-methoxy-1,2-benzothiazol-3(2H)-one

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The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of 3,3-dioxido-4-methoxy-1,2-benzothiazol-3(2H)-one prepared as described by D. J. Hlasta, J. J. Court and R. C. Desai, *Tetrahedron Lett.* **1991**, *32*, 7179-82, 1 mL of N,N-dimethylformamide and 0.1

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g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

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EXAMPLE 72**2-(4-Bromobutyl)-1,1-dioxido-6-methoxy-1,2-benzothiazol-3(2H)-one**

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of 3,3-dioxido-6-methoxy-1,2-benzothiazol-3(2H)-one prepared from N,N-diethyl-4-methoxybenzamide using the general procedure described by D. J. Hlasta, J. J. Court and R. C. Desai, *Tetrahedron Lett.* **1991**, *32*, 7179-82, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

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EXAMPLE 73**2-(4-Bromobutyl)-1,1-dioxido-7-methoxy-1,2-benzothiazol-3(2H)-one**

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of

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3,3-dioxido-7-methoxy-1,2-benzothiazol-3(2H)-one prepared from N,N-diethyl-3-methoxybenzamide using the general procedure described by D. J. Hlasta, J. J. Court and R. C. Desai, *Tetrahedron Lett.* **1991**, 32, 7179-82, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

EXAMPLE 74

2-(4-Bromobutyl)-1,1-dioxido-5-methyl-1,2-benzothiazol-3(2H)-one

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, 21, 583-4 was modified: A mixture of 0.5 g of 3,3-dioxido-5-methyl-1,2-benzothiazol-3(2H)-one prepared as described by J. G. Lombardino, *J. Org. Chem.* **1971**, 36, 1843-5, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

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EXAMPLE 75**2-(4-Bromobutyl)-1,1-dioxido-5-fluoro-1,2-benzothiazol-3(2H)-one**

5 The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of 3,3-dioxido-5-fluoro-1,2-benzothiazol-3(2H)-one prepared as described by J. G. Lombardino, *J. Org. Chem.* **1971**, *36*, 1843-5, 1
10 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure
15 and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

EXAMPLE 76

20 **2-(4-Bromobutyl)-1,1-dioxido-5-chloro-1,2-benzothiazol-3(2H)-one**

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of
25 3,3-dioxido-5-chloro-1,2-benzothiazol-3(2H)-one prepared as described by J. G. Lombardino, *J. Org. Chem.* **1971**, *36*, 1843-5, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient
30 temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which

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was free of 1,4-dibromobutane by ^1H -NMR and was used without further purification.

EXAMPLE 77

5 **2-(4-Bromobutyl)-1,1-dioxido-6-nitro-1,2-benzothiazol-3(2H)-one**

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A stirred mixture of 1.0
10 g of 3,3-dioxido-6-nitro-1,2-benzothiazol-3(2H)-one prepared as described by H. Kamogawa, S. Yamamoto and M. Nanasawa, *Bull. Chem. Soc. Japan*, **1982**, *55*, 3824-7, and 6 mL of 1N NaOH was warmed to dissolution and allowed to cool to ambient temperature for
15 15 min. The mixture was concentrated to dryness under reduced pressure and the white solid azeotropically dried with 20 mL of toluene. The resulting sodium salt was dissolved in 3 mL of N,N-dimethylformamide and 2.6 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was
20 diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO_3 , 25 mL of sat'd brine and dried over MgSO_4 . Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a solid which was free of 1,4-dibromobutane by ^1H -NMR and was used without further purification.

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EXAMPLE 78

2-(4-Bromobutyl)-1,1-dioxido-2,3-dihydronaphth[1,8-de]isothiazin-3-one

The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of
30 1,1-dioxido-2,3-dihydronaphth[1,8-de]isothiazin-3-one prepared as described by J. G. Lombardino, *J. Org. Chem.* **1971**, *36*, 1843-5, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL

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of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

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EXAMPLE 79**2-(4-Bromobutyl)-3,3-dioxido-1,2-dehydronaphth[1,2-d]isothiazol-1-one**

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The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of 3,3-dioxido-1,2-dehydronaphth[1,2-d]isothiazol-1-one prepared as described by J. G. Lombardino, *J. Org. Chem.* **1971**, *36*, 1843-5, 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride 60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

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EXAMPLE 80**2-(4-Bromobutyl)-pyrrolo[3,4b]pyridin-5,7(1H)-dione**

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The general procedure of J. D. Commerford and H. B. Donahue *J. Org. Chem.* **1956**, *21*, 583-4 was modified: A mixture of 0.5 g of commercial pyrrolo[3,4b]pyridin-5,7(1H)-dione (Aldrich Chemical Co.), 1 mL of N,N-dimethylformamide and 0.1 g of sodium hydride

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60% oil dispersion was stirred at ambient temperature for 15 min, and 1.4 mL of 1,4-dibromobutane was added. After stirring at ambient temperature overnight, the mixture was diluted with 25 mL of ethyl acetate, washed with 25 mL of sat'd NaHCO₃, 25 mL of sat'd brine
5 and dried over MgSO₄. Removal of solvents under reduced pressure and vacuum distillation gave a crude product as a viscous oil which was free of 1,4-dibromobutane by ¹H-NMR and was used without further purification.

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EXAMPLE 81

1,1-Dioxido-2-(4-(spiro[3-oxo-phthalan-1,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one

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From spiro[phthalan-1,4'-piperidine]-3-one, prepared as described in US Patent 3,686,186, and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 226-8°C.

20

EXAMPLE 82

5-chloro-1-(4-piperidiny)-3-benzoxazolin-2-one

25

From the reaction of 2-amino-4-chlorophenol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

30

EXAMPLE 83

4-(3a-(R)-8a-(S)-2-oxo-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazolinyl)-piperidine

From the reaction of (-)-1(S)-amino-2(R)-hydroxyindan, prepared according to W. J. Thompson et. al. *J. Med. Chem.* 1992, 35, 1685-1701, and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

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EXAMPLE 84**4-(2-oxonaphth[2,3-d]oxazoliny)-piperidine**

5 From the reaction of 3-amino-2-naphthol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 85**4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidine**

10 From the reaction of 6-amino-m-cresol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 86**4-(2-oxo-5-phenyl-3-benzoxazoliny)-piperidine**

15 From the reaction of 2-amino-4-phenylphenol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 87

20 **4-(6-methoxy-2-oxo-3-benzoxazoliny)-piperidine**

From the reaction of 2-amino-5-methoxyphenol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

25

EXAMPLE 88**4-(6-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidine**

From the reaction of methyl 4-amino-3-hydroxybenzoate and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

30

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EXAMPLE 89**4-(5-ethylsulfonyl-2-oxo-3-benzoxazoliny)-piperidine**

5 From the reaction of 2-amino-4-(ethylsulfonyl)phenol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 90**4-(2-oxo-3-oxazolo[4,5-b]pyridyl)-piperidine**

10 From the reaction of 2-amino-3-hydroxypyridine and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 91**4-(7-carbethoxy-2-oxo-3-benzoxazoliny)-piperidine**

15 From the reaction of methyl 3-amino-2-hydroxybenzoate and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 92**4-(5-tert-butyl-2-oxo-3-benzoxazoliny)-piperidine**

20 From the reaction of 2-amino-4-tert-butylphenol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

EXAMPLE 93**4-(5,7-dimethyl-2-oxo-3-benzoxazoliny)-piperidine**

25 From the reaction of 6-amino-2,4-dimethylphenol and N-t-butyloxycarbonyl-4-piperidone according to Example 15 Steps 2-4 was obtained a white solid.

30

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EXAMPLE 94**1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

- 5 From 1,3-dihydro-1-(4-piperidinyl)-2H-benzimidazol-2-one and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 193-5°C; Analysis calculated for $C_{23}H_{26}N_4O_4S \cdot 0.5 CHCl_3$ C: 54.89 H: 5.19, N: 10.90 found C: 54.60, H: 5.24, N: 10.84. 1H NMR (300 MHz, $CDCl_3$) 10.0 (s, 1H), 8.2 (d, 1H), 8.1-7.9 (m, 3H), 7.4 (dd, 1H), 7.25 (m, 1H), 7.18 (m, 3H), 4.5 (m, 1H), 3.95 (t, 2H), 3.2 (d, J = 12 Hz, 2H), 2.6 (m, 4H), 2.3 (t, J = 12 Hz, 2H), 2.1 (m, 4H), 1.95 (m, 2H), 1.8(m, 2H).

15

EXAMPLE 95**1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-3-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

- 20 Step 1: A mixture of 1 g of 1,3-dihydro-1-(4-piperidinyl)-2H-benzimidazol-2-one, 25 mL of tetrahydrofuran, 10 mL of saturated Na_2CO_3 , 20 mL of water and 1.05 g of di-tert-butyl-dicarbonate was stirred for 6 h. The mixture was extracted with 100 mL of chloroform and the organic extracts concentrated to dryness under reduced pressure. Drying under vacuum gave 1.6 g of 1,3-dihydro-1-(1-tert-butyloxycarbonylpiperidin-4-yl)-2H-benzimidazol-2-one as a white crystalline solid.
- 25 Step 2: A mixture of 0.060 g of sodium hydride, 60% oil dispersion, 10 mL of DMF, 0.086 mL of iodomethane, and 0.4 g of 1,3-dihydro-1-(1-tert-butyloxycarbonylpiperidin-4-yl)-2H-benzimidazol-2-one was stirred for 12 h, then partitioned between 100 mL of chloroform and 50 mL of water. The chloroform extracts dried over $MgSO_4$, and concentrated under reduced pressure. Trituration with hexane gave 0.38 g of 1,3-dihydro-1-(1-tert-butyloxycarbonylpiperidin-4-yl)-3-
- 30

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methyl-2H-benzimidazol-2-one as a crystalline solid: ^1H NMR (300 MHz, CDCl_3) 7.13-7.03 (m, 3H), 6.98 (d, 1H), 4.5 (m, 1H), 4.3 (br m, 2H), 3.4 (s, 3H), 2.85 (m, 4H), 2.3 (m, 2H), 1.8 (d, 2H), 1.5 (s, 9H).

- 5 Step 3: A stream of hydrogen chloride gas was dispersed through a stirred, ice cold solution of 0.383 g of 1,3-dihydro-1-(1-tert-butylloxycarbonylpiperidin-4-yl)-3-methyl-2H-benzimidazol-2-one in 500 mL of ethyl acetate for 30 min. Stirring was continued at 0°C for 1 h, then at ambient temperature for 1 h. The suspension was
- 10 partitioned between 250 mL of chloroform and 50 mL of saturated Na_2CO_3 . Drying under reduced pressure gave 0.296 of 1,3-dihydro-1-(4-piperidinyl)-2H-benzimidazol-2-one as an off-white solid.
- 15 Step 4: From 1,3-dihydro-1-(4-piperidinyl)-3-methyl-2H-benzimidazol-2-one and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp $161-163^\circ\text{C}$. Analysis calculated for $\text{C}_{24}\text{H}_{28}\text{N}_4\text{O}_4\text{S}$ C: 61.52, H: 6.02, N: 11.96 found C: 61.44, H: 6.06, N: 11.84. ^1H NMR (300 MHz, CDCl_3) 8.2 (d, 1H),
- 20 8.1-7.9 (m, 3H), 7.13-7.03 (m, 3H), 6.98 (d, 1H), 4.5 (m, 1H), 3.95 (t, 2H), 3.4 (s, 3H), 3.2 (d, $J = 12$ Hz, 2H), 2.6 (m, 4H), 2.3 (t, $J = 12$ Hz, 2H), 2.1 (m, 4H), 1.95 (m, 2H), 1.8(m, 2H).

EXAMPLE 96

- 25 **1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

- 30 Step 1: A mixture of 69 g of 4-chloro-3-nitro-toluene, 50 g of ethyl 4-amino-1-piperidinecarboxylate, 24 g of sodium carbonate, 0.1 g of sodium iodide and 120 mL of cyclohexanol was heated to 150°C for 72 h. After cooling the cyclohexanol was distilled off under reduced pressure and the residue partitioned between 1 L of ethyl acetate and 1 L of water. The organic extract was dried over MgSO_4 and concentrated under reduced pressure. Chromatography over silica gel,

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eluting with 20% ethyl acetate in cyclohexane gave 38.5 g (42.3%) of ethyl 4-(4-methyl-2-nitroanilino)-1-piperidinecarboxylate as an orange crystalline solid. ¹H NMR (400 MHz, CDCl₃) 8.0 (s, 1H), 7.27 (t, J = 9 Hz, 1H), 6.8 (d, J = 9 Hz, 1H), 4.15 (q, J = 7 Hz, 2H), 4.05 (br m, 2H), 3.67 (br m, 1H), 3.10 (br t, J = 11 Hz, 2H), 2.27 (s, 3H), 2.06 (br d, J = 11 Hz, 2H), 1.6 (m, 2H), 1.27 (t, J = 7 Hz, 4H).

Step 2: A mixture of 8.23 g of ethyl 4-(4-methyl-2-nitroanilino)-1-piperidinecarboxylate, 200 mL of tetrahydrofuran, 225 mL of ethanol and 2 g of 5% platinum on carbon was stirred under an atmosphere of hydrogen for 7 h. The catalyst was filtered off and the filtrate concentrated to a thick oil. To an ice cold, vigorously stirred solution of the resulting crude ethyl 4-(4-methyl-2-aminoanilino)-1-piperidinecarboxylate in 500 mL of ethyl acetate was added 500 mL of saturated sodium carbonate followed by 20 mL of 1.9 M phosgene in toluene dropwise over 30 min. After stirring overnight at room temperature, the layers were separated and the organic layer dried over MgSO₄ and concentrated to dryness. Trituration of the residue with ether-hexane gave 8 g of ethyl 4-(5-methyl-2-oxo-1-benzimidazoliny)l)piperidine-1-carboxylate as a white crystalline solid.

Step 3: A mixture of 5 g of ethyl 4-(5-methyl-2-oxo-1-benzimidazoliny)l)piperidine-1-carboxylate and 20 mL of 2N NaOH was heated under reflux for 12 h. The resulting solution is cooled and stirred for 30 minutes with 5 g of ammonium chloride and extracted with three 200 mL portions of chloroform. The combined organic extracts were dried over MgSO₄, concentrated under reduced pressure and triturated with ether. The solid product 1,3-dihydro-1-(4-piperidinyl)-5-methyl-2H-benzimidazol-2-one weighed 3.5 g after drying.

Step 4: From 1,3-dihydro-1-(4-piperidinyl)-5-methyl-2H-benzimidazol-2-one and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 104-6°C; Analysis calculated for C₂₄H₂₈N₄O₄S•0.35 CH₃OH•0.35 CH₂Cl₂ C: 58.22, H: 5.95, N: 11.00 found C: 58.43, H: 5.91, N: 10.60.

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EXAMPLE 97

5 **1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-4-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

From 1,3-dihydro-1-(4-piperidinyl)-4-methyl-2H-benzimidazol-2-one and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white
10 solid: mp 90-2°C; Analysis calculated for C₂₄H₂₈N₄O₄S•0.5 CH₂Cl₂ C: 57.58, H: 5.72, N: 10.96 found C: 57.41, H: 5.74, N: 10.96.

EXAMPLE 98

15 **1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methoxy-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

From 4-chloro-3-nitro-anisole using the procedures described for Example 96, Steps 1 through 4, there was obtained a white solid: mp
20 162-4°C; Analysis calculated for C₂₄H₂₈N₄O₅S•0.5 CH₃OH C: 58.78, H: 6.04, N: 11.19 found C: 58.99, H: 5.88, N: 10.87.

EXAMPLE 99

25 **1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-chloro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

From 2,5-dichloronitrobenzene using the procedures described for Example 96, Steps 1 through 4, there was obtained a white solid: mp
30 206-8°C; Analysis calculated for C₂₃H₂₅ClN₄O₄S•0.8 CH₂Cl₂ C: 54.46, H: 5.11, N: 10.68 found C: 54.34, H: 4.87, N: 10.81.

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EXAMPLE 100

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-fluoro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one

5

From 2-chloro-5-fluoronitrobenzene using the procedures described for Example 96, Steps 1 through 4, and 2-(4-bromobutyl)-1,1-dioxido-5-chloro-1,2-benzisothiazol-3(2H)-one there was obtained a white solid: mp 259-61°C; Analysis calculated for C₂₃H₂₄FCIN₄O₄S • HCl C: 50.83, H: 4.64, N: 10.31 found C: 50.74, H: 4.60, N: 10.18.

10

EXAMPLE 101

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one

15

From 1,3-dihydro-1-(4-piperidinyl)-5-methyl-2H-benzimidazol-2-one and 2-(4-bromobutyl)-1,1-dioxido-5-chloro-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 258-60°C; Analysis calculated for C₂₃H₂₇ClN₄O₄S • HCl • 0.4 H₂O C: 52.72, H: 5.31, N: 10.25 found C: 52.75, H: 5.23, N: 10.25.

20

EXAMPLE 102

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-6-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one

25

From 1,3-dihydro-1-(4-piperidinyl)-5-methyl-2H-benzimidazol-2-one and 2-(4-bromobutyl)-1,1-dioxido-5-chloro-1,2-benzisothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: mp 273-75°C; Analysis calculated for C₂₃H₂₇ClN₄O₄S • HCl • 0.5 H₂O C: 52.55, H: 5.33, N: 10.22 found C: 52.52, H: 5.05, N: 10.16.

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EXAMPLE 103

5 **1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazolinyl)-**
piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-
e]benzisothiazol-3(2H)-one

From 4-(5-methyl-2-oxo-3-benzoxazolinyl)-piperidine and 2-(4-
hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-
10 d]benzisothiazol-3(2H)-one using the procedure described for Example
66, Steps 4-6 there was obtained a white solid: mp 98-101 °C

Analysis calculated for C₂₆H₂₉N₃O₇S • 0.3 CHCl₃ C: 56.06, H:
5.24, N: 7.46 found C: 56.11, H: 5.30, N: 7.45. ¹H NMR (300 MHz,
CDCl₃) 7.4 (d, H), 7.27 (d, 1H), 7.18 (d, 1H), 7.05 (s, 1H), 6.95 (d,
1H), 4.5 (m, 2H), 4.4 (m, 2H), 4.2 (m, 1H), 3.8 (t, 2H), 3.08 (d, 2H),
15 2.44 (t, 2H), 2.39 (s, 3H), 2.1 (t, 2H), 1.85 (m, 4H), 1.71 (m, 2H),
1.65 (m, 2H).

20

25

30

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EXAMPLE 104

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one

5

From 4-(2-oxo-3-benzoxazoliny)-piperidine and 2-(4-hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-d]benzisothiazol-3(2H)-one using the procedure described for Example 66, Steps 4-6 there was obtained a white solid: mp 79-82 °C ¹H NMR (400 MHz, CDCl₃) 7.4 (d, 1H), 7.3 (m, 2H), 7.2-7.0 (br m, 3H), 4.5 (m, 2H), 4.4 (m, 2H), 4.2 (m, 1H), 3.8 (m, 2H), 3.1 (br d, 2H), 2.5-2.4 (br m, 2H), 2.2 (m, 2H), 2.0-1.85 (br m, 4H), 1.80 (m, 2H), 1.7 (m, 2H) Analysis calculated for C₂₅H₂₇N₃O₇S • 0.3 CHCl₃ C: 55.31, H: 5.01, N: 7.65 found C: 55.20, H: 5.08, N: 7.39.

15

EXAMPLE 105

1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one

20

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-d]benzisothiazol-3(2H)-one using the procedure described for Example 66, Steps 4-6 there was obtained a white solid: Analysis calculated for C₂₆H₂₉N₂O₆S • 0.4 CHCl₃ C: 58.25, H: 5.26, N: 5.15 found C: 58.36, H: 5.40, N: 5.20.

25

EXAMPLE 106

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-1H-3,4-dihydroquinazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

30

Step 1: A mixture of 45 g of 2-aminomethylaniline, 60 g of di-*tert*-butyldicarbonate, 1000 mL of dichloromethane was stirred for 18 h and washed with 500 mL of 2N NaOH. The organic extract was dried

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over MgSO₄ and concentrated under reduced pressure. Drying under vacuum gave 47 g of 2-(*tert*-butoxycarbonylaminomethyl)aniline as a white crystalline solid. ¹H NMR (400 MHz, CDCl₃) 7.1 (t, 1H), 7.05 (d, 1H), 6.65 (dd, 2H), 4.8 (br s, 1H), 4.2 (br m, 4H), 1.44 (s, 9H).

5 Step 2: A mixture of 15.5 g of 2-(*tert*-butoxycarbonylaminomethyl)aniline, 15 g of N-t-butyloxycarbonyl-4-piperidone, 250 mL of 1,2-dichloroethane, 4.2 mL of glacial acetic acid and 25 g of sodium triacetoxymethylborohydride was stirred at room temperature for 48 h. The reaction mixture was poured into 500 mL chloroform and 500 mL saturated aqueous Na₂CO₃ and the layers separated. The aqueous layer was extracted with 2 X 250 mL of chloroform and the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. Drying overnight under vacuum gave 30.1 g of *tert*-butyl 2-(*tert*-butoxycarbonylaminomethyl)anilino)piperidine carboxylate as a solid: ¹H NMR (400 MHz, CDCl₃) 7.19 (t, 1H), 7.0 (d, 1H), 6.6 (dd, 2H), 4.94 (br s, 1H), 4.75 (br s, 1H), 4.23 (br m, 2H), 4.0 (br m, 2H), 3.45 (br m, 1H), 3.0 (br m, 2H), 2.0 (br m, 2H), 1.82 (br m, 1H), 1.46 (s, 9H), 1.44 (s, 9H).

20 Step 3: To a stirred solution of 27.1 g of *tert*-butyl 4-(2-(*tert*-butoxycarbonylaminomethyl)anilino)-1-piperidinecarboxylate and 30 mL of triethylamine in 400 mL of dichloromethane was added dropwise 60 mL of a 1.93 M solution of phosgene in toluene. After stirring for 12 h, 200 mL of 1N NaOH was added. The mixture was shaken, and the organic layer separated, dried over MgSO₄ and concentrated under reduced pressure. Chromatography on silica gel, eluting with 25% ethyl acetate in hexane gave, after drying overnight under vacuum, 25 g of 1,3-dihydro-1-[1-(*tert*-butoxycarbonylpiperidin-4-yl)-3-(*tert*-butoxycarbonyl)-1H-3,4-dihydroquinazolin-2-one carboxylate as a clear glass: ¹H NMR (400 MHz, CDCl₃) 7.52 (d, 1H), 7.45 (t, 1H), 7.36 (m, 1H), 7.10 (d, 1H), 4.2-4.0 (br m, 5H), 3.65-3.25 (br m, 2H), 2.75 (br m, 2H), 2.28 (br d, 1H), 1.8 (br d, 1H), 1.5 (s, 9H), 1.49 (s, 9H).

30 Step 4: A stirred solution of 25 g of 1,3-dihydro-1-[1-(*tert*-butoxycarbonylpiperidin-4-yl)-3-(*tert*-butoxycarbonyl)-1H-3,4-dihydroquinazolin-2-one carboxylate in 1 L of ethyl acetate cooled to

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-50°C was saturated with hydrogen chloride gas for 15 min. The resulting mixture was allowed to warm to room temperature and stir for 4 h. The white solid precipitate was collected by filtration. Drying under vacuum gave 13.1 g of 1,3-dihydro-1-[piperidin-4-yl]-3-*tert*-butoxycarbonyl-1H-3,4-dihydroquinazolin-2-one hydrochloride salt as a white solid. The salt (0.8 g) was converted to the free base by partitioning between chloroform and saturated sodium carbonate. Drying under vacuum gave 0.68 g of 1,3-dihydro-1-[piperidin-4-yl]-1H-3,4-dihydroquinazolin-2-one as a white solid: ¹H NMR (400 MHz, CDCl₃) 7.25 (dd, 1H), 7.12 (d, 1H), 7.06 (d, 1H), 6.98 (t, 1H), 5.2 (br s, 1H), 4.28 (s, 2H), 4.10 (m, 1H), 3.22 (d, 2H), 2.73 (m, 2H), 2.59 (m, 2H), 2.05 (br s, 1H), 1.82 (br d, 2H).
Step 5: From 1,3-dihydro-1-[piperidin-4-yl]-1H-3,4-dihydroquinazolin-2-one and 2-(4-bromobutyl)-1,1-dioxido-1,2-benzothiazol-3(2H)-one using the procedure described for Example 15, Step 5 there was obtained a white solid: Analysis calculated for C₂₄H₂₈N₄O₄S • 0.35 CHCl₃ • 0.45 H₂O C: 52.70 H: 5.49, N: 10.10 found C: 52.71, H: 5.49, N: 10.33.

EXAMPLE 107

2-(4-Hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-d]benzisothiazol-3(2H)-one

Step 1: To a stirred solution of 12 g of 1,4-benzodioxane in 40 mL of chloroform cooled to -10°C under calcium sulfate drying tube was added dropwise over 15 min, 6 mL of chlorosulfonic acid. The mixture was allowed to warm to 20°C over 15 min, poured onto 400 mL of ice and extracted into 1 L of chloroform. Drying over MgSO₄ and concentration under reduced pressure gave 21.5 g of 1,4-benzodioxane-6-sulfonylchloride as a white crystalline solid.

Step 2: To an ice cold, stirred solution of 10 g of 4-aminobutanol and 25 mL of triethylamine in 250 mL of dichloromethane was added a solution of 21.5 g of 1,4-benzodioxane-6-sulfonylchloride in 100 mL of chloroform over 30 min. The mixture was allowed to warm and stir overnight, washed with 100 mL of 6N HCl and dried over MgSO₄. To

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this solution was added 300 mL of dichloromethane, 20 mL of dihydropyran and 100 mg of p-toluenesulfonic acid monohydrate. After stirring for 12 h, the mixture was washed with 100 mL of saturated Na₂CO₃, dried over MgSO₄ and concentrated under reduced
5 pressure. Chromatography on silica gel, eluting with 30% ethyl acetate in hexane gave, after drying overnight under vacuum, 13 g of N-(4-(2-tetrahydropyranyloxy)butyl)-1,4-benzodioxane-6-sulfonamide as a resin.

Step 3: To a stirred solution of 13 g of N-(4-(2-tetrahydropyranyloxy)butyl)-1,4-benzodioxane-6-sulfonamide in 250
10 mL of dry tetrahydrofuran cooled to -78°C was added 50 mL of commercial 1.6M n-butyllithium in hexane over 10 min. After aging for 60 min with warming to 0°C, the solution was cooled to -78°C and quenched by a stream of carbon dioxide gas with a fritted glass
15 dispersion tube, keeping the temperature below -40°C for 30 min. The solution was allowed to warm to room temperature over 1 h and partitioned between 200 mL of water, 200 mL of saturated Na₂CO₃ and 3 x 200 mL of ether. The aqueous layer was carefully acidified to pH=3 with concentrated HCl, and extracted with 3 x 200 mL portions
20 of ether. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. Drying under vacuum gave 10.1 g of N-(4-(2-tetrahydropyranyloxy)butyl) 5-carboxy-1,4-benzodioxane-6-sulfonamide as a foam.

Step 4: To a stirred solution of 10 g of N-(4-(2-tetrahydropyranyloxy)butyl) 5-carboxy-1,4-benzodioxane-6-sulfonamide and 7 mL of triethylamine in 200 mL of dichloromethane cooled to 0°C was added 2 mL of methyl chloroformate dropwise.
25 After 3 h at 20°C, the mixture was shaken with 50 mL of saturated sodium carbonate, dried over MgSO₄ and concentrated under reduced pressure. The residue was dissolved in 200 mL of methanol containing
30 1 g of p-toluenesulfonic acid monohydrate, stirred overnight at ambient temperature, and concentrated to dryness under reduced pressure. The residue was dissolved in 200 mL of ethyl acetate, washed with 5 mL of saturated sodium carbonate, dried over MgSO₄

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and concentrated under reduced pressure. Trituration with ether gave 4 g of 2-(4-hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-d]benzothiazol-3(2H)-one as a white crystalline solid: mp °C; ¹H NMR (400 MHz, CDCl₃): 7.4 (d, 1H), 7.25 (d, 1H), 4.5 (m, 2H), 4.4 (m, 2H), 3.8 (t, 2H), 3.7 (t, 2H), 1.95 (m, 2H), 1.7 (m, 2H), 1.55 (br s, 1H).

EXAMPLE 108

2-(4-Hydroxybutyl)-1,1-dioxido-5-ethoxy-1,2-benzothiazol-3(2H)-one

From ethoxybenzene using the procedures described for Example 107, Steps 1-4 there was obtained a thick oil.

EXAMPLE 109

2-(4-Hydroxybutyl)-1,1-dioxido-5-ethyl-1,2-benzothiazol-3(2H)-one

From 4-ethylbenzenesulfonylchloride using the procedures described for Example 107, Steps 2-4 there was obtained a thick oil.

EXAMPLE 110

2-(4-Hydroxybutyl)-1,1-dioxido-5-isopropyl-1,2-benzothiazol-3(2H)-one

From 4-isopropylbenzenesulfonylchloride using the procedures described for Example 107, Steps 2-4 there was obtained a thick oil.

EXAMPLE 111

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-ethoxy-1,2-benzothiazol-3(2H)-one

From 4-(2-oxo-3-benzoxazolinyl)-piperidine and 2-(4-hydroxybutyl)-1,1-dioxido-5-ethoxy-1,2-benzothiazol-3(2H)-one using the procedure

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described for Example 66, Steps 4-6 there was obtained a white solid:
¹H NMR (400 MHz, CDCl₃) 7.79 (d, J = 8.57 Hz, 1H), 7.44 (s, 1H),
7.29-7.26 (m, 2H), 7.21 (d, J = 6.89 Hz, 1H), 7.16-7.06 (m, 2H), 4.24-
4.11 (m, 1H), 3.81 (t, J = 7.3 Hz, 2H), 3.06 (br d, J = 11.76 Hz, 2H),
2.45 (t, J = 7.39 Hz, 2H), 2.36 (m, 2H), 2.11 (bt, J = 11.08 Hz, 2H),
1.93-1.80 (br m, 6H), 1.65 (br m, 2H), 1.48 (t, J = 6.97, 3H) Analysis
calculated for C₂₅H₃₀N₃O₆S • HCl • 1.2 H₂O C: 53.75, H: 6.03, N:
7.52 found C: 53.72, H: 5.65 N: 7.52.

10

EXAMPLE 112

**1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-
2,4'-piperidin-1'-yl)-butyl)-5-ethoxy-1,2-benzothiazol-
3(2H)-one**

15

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-
hydroxybutyl)-1,1-dioxido-5-ethoxy-1,2-benzothiazol-3(2H)-one using
the procedure described for Example 66, Steps 4-6 there was obtained a
white solid: Analysis calculated for C₂₆H₃₀N₂O₅S • HCl • 0.45 H₂O •
0.3 CHCl₃ C: 56.11, H: 5.77, N: 4.98 found C: 56.14, H: 5.74, N:
5.18.

20

EXAMPLE 113

25

**1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-
2,4'-piperidin-1'-yl)-butyl)-5-ethyl-1,2-benzothiazol-3(2H)-
one**

30

From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-
hydroxybutyl)-1,1-dioxido-5-ethyl-1,2-benzothiazol-3(2H)-one using
the procedure described for Example 66, Steps 4-6 there was obtained a
white solid: Analysis calculated for C₂₆H₃₀N₂O₄S • HCl • 0.2 CHCl₃
C: 59.71, H: 5.97, N: 5.32 found C: 59.66, H: 6.16, N: 5.35.

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EXAMPLE 114

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-ethyl-1,2-benzothiazol-3(2H)-one

5 From 4-(2-oxo-3-benzoxazoliny)-piperidine and 2-(4-hydroxybutyl)-1,1-dioxido-5-ethyl-1,2-benzothiazol-3(2H)-one using the procedure described for Example 66, Steps 4-6 there was obtained a white solid: mp 165-168 °C ¹H NMR (400 MHz, CDCl₃) 7.9 (s, 1H), 7.8 (m, 1H), 7.66 (m, 1H), 7.3 (m, 1H), 7.2-7.05 (m, 3H), 4.2 (m, 1H), 3.8 (m,
10 2H), 3.1 (m, 2H), 2.82 (m, 2H), 2.4 (m, 2H), 2.1 (m, 2H), 2.0-1.8 (br m, 4H), 1.8-1.6 (br m, 4H), 1.3 (t, 3H) Analysis calculated for C₂₅H₃₀N₃O₆S • HCl • 1.4 H₂O C: 55.66, H: 6.06, N: 7.01 found C: 55.82, H: 5.81, N: 6.91.

15

EXAMPLE 115

1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-(2-propyl)-1,2-benzothiazol-3(2H)-one

20 From 1,3-dihydro-1-oxo-spiro(2H-indenyl-2,4'-piperidine) and 2-(4-hydroxybutyl)-1,1-dioxido-5-(2-propyl)-1,2-benzothiazol-3(2H)-one using the procedure described for Example 66, Steps 4-6 there was obtained a white solid: Analysis calculated for C₂₇H₃₂N₂O₄S • HCl • 1.00 H₂O C: 60.60, H: 6.59, N: 5.24 found C: 60.62, H: 6.36, N: 5.28.

25

EXAMPLE 116

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-(2-propyl)-1,2-benzothiazol-3(2H)-one

30 From 4-(2-oxo-3-benzoxazoliny)-piperidine and 2-(4-hydroxybutyl)-1,1-dioxido-5-(2-propyl)-1,2-benzothiazol-3(2H)-one using the procedure described for Example 66, Steps 4-6 there was obtained a white solid: ¹H NMR (400 MHz, CDCl₃) 7.9 (s, 1H), 7.8 (d, 1H), 7.7 (d, 1H), 7.3 (m, 1H), 7.2-7.0 (br m, 3H), 4.2 (m, 1H), 3.8 (m, 2H),

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3.1 (m, 3H), 2.7-2.5 (br m, 2H), 2.1 (m, 2H), 2.0 - 1.8 (br m, 4H),
1.7-1.6 (m, 4H), 1.35 (d, 6H) Analysis calculated for C₂₅H₃₀N₃O₆S
• HCl • 1.6 CH₃CO₂CH₂CH₃ C: 57.64, H: 6.69, N: 6.23 found C:
57.06, H: 6.12, N: 6.56.

5

The following Examples 117-144 were made in the same
manner as described in detail above using readily available starting
materials.

10

EXAMPLE 117

**1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-
yl)-butyl)-6-nitro-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 8.78 (d, J = 1.46 Hz, 1H), 8.70 (dd, J =
15 8.39 Hz, 2.01 Hz, 1H), 8.29 (dd, J = 8.39 Hz, 0.4 Hz, 1H), 7.26-7.08
(m, 4H), 4.21 (dt, J = 12.43 Hz, 4.2 Hz, 1H), 3.91 (t, J = 7.40 Hz, 2H),
3.08 (d, J = 11.58 Hz, 2H), 2.47 (t, J = 7.22 Hz, 2H), 2.39 (dq, J =
12.43 Hz, 3.80 Hz, 2H), 2.12 (t, J = 10.24 Hz, 2H), 1.95 (q, J = 7.56 Hz,
20 2H), 1.87 (dd, J = 11.92 Hz, 2.01 Hz, 2H), 1.65 (q, J = 7.89 Hz, 2H).

20

EXAMPLE 118

**1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-
yl)-butyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 7.95 (dd, J = 4.37 Hz, 4.20 Hz, 1H), 7.73
(dd, J = 7.05 Hz, 2.35 Hz, 1H), 7.56 (dt, J = 8.39 Hz, 2.52 Hz, 1H), 7.25
(s, 1H), 7.20 (d, J = 7.89 Hz, 1H), 7.14 (dt, J = 7.55 Hz, 1.18 Hz, 1H),
7.09 (dq, J = 7.72 Hz, 1.18 Hz, 1H), 4.22 (tt, J = 12.42 Hz, 4.2 Hz, 1H),
3.84 (t, J = 7.39 Hz, 2H), 3.07 (d, J = 11.75 Hz, 2H), 2.45 (t, J = 7.22
25 Hz, 2H), 2.38 (dq, J = 8.70 Hz, 3.90 Hz, 2H), 2.12 (dt, J = 11.75 Hz,
30 1.51 Hz, 2H), 1.82 - 1.60 (m, 4H), 1.64 (q, J = 7.56 Hz, 2H).

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EXAMPLE 119

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-methyl-1,2-benzisothiazol-3(2H)-one

5 ¹H NMR (400 MHz, CDCl₃) 7.86 (s, 1H), 7.81 (d, J = 7.89 Hz, 1H), 7.66 (d, J = 8.06 Hz, 1H), 7.21 (d, J = 7.55 Hz, 1H), 7.15 (t, J = 6.21 Hz, 1.51 Hz, 1H), 4.2 (m, 1H), 3.83 (t, J = 7.39 Hz, 2H), 3.08 (d, J = 11.75 Hz, 2H), 2.56 (s, 3H), 2.45 (t, J = 6.88 Hz, 2H), 2.37 (dt, J = 8.22 Hz, 3.86 Hz, 2H), 2.11 (t, J = 12.26 Hz, 2H), 1.94 - 1.85 (m, 4H), 1.63 (m, 2H).
10

EXAMPLE 120

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-bromo-1,2-benzisothiazol-3(2H)-one

15 mp 158-162 °C ¹H NMR (400 MHz, CDCl₃) 8.20 (m, 1H), 7.99 (dd, 1H), 7.79 (d, 1H), 7.22 - 7.06 (m, 4H), 4.21 (m, 1H), 3.83 (t, 2H), 3.07 (d, 2H), 2.55 (t, 2H), 2.42-2.30 (m, 2H), 2.10 (t, 2H), 1.94-1.82 (m, 4H), 1.61 (m, 2H).
20

EXAMPLE 121

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-trifluoromethyl-1,2-benzisothiazol-3(2H)-one

25 mp 177-179 °C ¹H NMR (400 MHz, CDCl₃) 8.38 (s, 1H), 8.18 (d, 1H), 8.10 (d, 1H), 7.25 (m, 1H), 7.2 (d, 1H), 7.1-7.2 (m, 2H), 4.2 (m, 1H), 3.88 (m, 2H), 3.05 (br d, 2H), 2.45 (m, 2H), 2.4 (m, 2H), 2.1 (m, 2H), 1.82-2.0 (br m, 4H), 1.75-1.6 (m, 2H).
30

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EXAMPLE 122

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-trifluoromethoxy-1,2-benzisothiazol-3(2H)-one

5 mp 230-233 °C ¹H NMR (400 MHz, CDCl₃) 8.0 (d, 1H), 7.9 (s, 1H), 7.68 (d, 1H), 7.25 (d, 1H), 7.20 (d, 1H), 7.1-7.19 (m, 2H), 4.2 (m, 1H), 3.83 (m, 2H), 3.05 (br d, 2H), 2.42 (m, 2H), 2.4 (m, 2H), 2.1 (m, 2H), 2.8-1.8 (br m, 4H), 1.6 (m, 2H).

10

EXAMPLE 123

1,1-Dioxido-2-(4-(4-(2-oxo-1-naphth[1,2-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

15 ¹H NMR (400 MHz, CD₃OD) 7.46 (d, J = 8.61 Hz, 1H), 7.24 (dd, J = 6.77, 0.73 Hz, 1H), 7.21 (d, J = 7.32 Hz, 1H), 7.16-7.09 (m, 3H), 6.90 (d, J = 8.79 Hz, 1H), 6.79 (t, J = 7.30 Hz, 1H), 6.66 (t, J = 7.51 Hz, 1H), 6.64 (d, J = 8.79 Hz, 1H), 4.35 (m, 1H), 3.03 (t, J = 6.22 Hz, 2H), 2.89 (d, J = 12.63 Hz, 2H), 2.63 (q, J = 6.96 Hz, 1H), 2.53 (br t, J = 12.45 Hz, 1H), 2.41 (br m, 2H), 2.17 (br m, 2H), 1.48 (br d, J = 13.55 Hz, 2H), 1.10 (br m, 4H).

20

EXAMPLE 124

1,1-Dioxido-2-(4-(4-(5,6,7,8-tetrahydro-2-oxo-3-naphth[2,3-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

25

30 ¹H NMR (300 MHz, CD₃OD) 8.09 (m, 2H), 7.98 (m, 2H), 7.08 (s, 1H), 6.93 (s, 1H), 3.87 (m, 2H), 3.74 (d, J = 12.21 Hz, 2H), 3.24 (br m, 4H), 2.79 (br m, 6H), 2.13 (br d, J = 13.54 Hz, 2H), 1.95 (br d, J = 3.47 Hz, 4H), 1.89 (s, 4H).

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EXAMPLE 125

1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

5

¹H NMR (400 MHz, CDCl₃) 8.08 (d, J = 7.05 Hz, 1H), 7.95-7.85 (m, 4H), 7.06 (d, J = 8.23 Hz, 1H), 6.92 (d, J = 8.22 Hz, 1H), 4.58 (br t, J = 11.92 Hz, 1H), 3.86 (t, J = 6.55 Hz, 2H), 3.72 (d, J = 10.58 Hz, 2H),
10 3.34 (q, J = 11.25 Hz, 2H), 3.13 (br m, 2H), 2.93 (br m, 2H), 2.45 (s, 3H), 2.11 - 1.99 (m, 6H).

EXAMPLE 126

1,1-Dioxido-2-(4-(4-(5-carbomethoxy-2-oxo-3-
benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-
15 **3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 8.63 (s, 1H), 8.09 (d, J = 7.05 Hz, 1H), 7.96-7.86 (m, 5H), 4.61 (br t, J = 13.09 Hz, 1H), 4.00 (s, 3H), 3.87
20 (t, J = 6.72 Hz, 2H), 3.75 (d, J = 10.91, 2H), 3.41 (q, J = 12.26, 2H), 3.13 (m, 2H), 2.93 (m, 2H), 2.13-2.00 (m, 6H).

EXAMPLE 127

1,1-Dioxido-2-(4-(4-(6-fluoro-2-oxo-3-benzoxazoliny)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

25

mp 154-155 °C ¹H NMR (400 MHz, CDCl₃) 8.06 (dd, 1H), 7.96-7.82 (m, 3H), 7.18 (br q, 1H), 6.99 (dd, 1H), 6.88 (td, 1H), 4.19 (m, 1H), 3.84 (t, 2H), 3.06 (d, 2H), 2.44 (t, 2H), 2.40-2.25 (m, 2H), 2.10 (t, 2H),
30 1.96-1.81 (m, 4H), 1.61 (bs, 2H).

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EXAMPLE 128

1,1-Dioxido-2-(4-(4-(4-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

5 ¹H NMR (400 MHz, CDCl₃) 8.07 (dd, J = 6.72, 1.52 Hz, 1H), 7.93 (d, J = 6.55 Hz, 1H), 7.85 (m, 2H), 7.03 (d, J = 7.05 Hz, 1H), 6.97 (t, J = 7.72 Hz, 1H), 6.89 (d, J = 7.72 Hz, 1H), 4.21 (dt, J = 8.23, 3.86 Hz, 1H), 3.86 (t, J = 7.21 Hz, 2H), 3.09 (d, J = 11.92 Hz, 2H), 2.71 (dq, J = 8.72, 3.7 Hz, 2H), 2.55 (s, 3H), 2.43 (t, J = 7.56 Hz, 2H), 2.03 (t, J = 12.08 Hz, 2H), 1.91 (m, 2H), 1.65 (d, J = 7.72 Hz, 2H), 1.63-1.59 (m, 2H).

EXAMPLE 129

15 **1,1-Dioxido-2-(4-(4-(4-methoxy-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 8.07 (dd, J = 5.37, 1.01 Hz, 1H), 7.94-7.82 (m, 3H), 7.04 (t, J = 8.22 Hz, 1H), 6.85 (dd, J = 7.21, 0.84 Hz, 1H), 6.73 (d, J = 8.56 Hz, 1H), 4.45 (tt, J = 12.25, 4.2 Hz, 1H), 3.94 (s, 3H), 20 3.84 (t, J = 7.39 Hz, 2H), 3.04 (br d, J = 11.58 Hz, 2H), 2.54 (qd, J = 8.73, 3.69 Hz, 2H), 2.43 (t, J = 7.22, 2H), 2.07 (dt, J = 10.24, 1.68 Hz, 2H), 1.93 (m, 2H), 1.77 (br d, J = 9.74 Hz, 2H), 1.63 (m, 2H).

EXAMPLE 130

25 **5-Chloro-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 8.04 (d, J = 1.68 Hz, 1H), 7.85 (overlapping t and dt, 2H), 7.13 (d, J = 8.05 Hz, 1H), 7.04 (s, 1H), 6.95 (d, J = 8.05 Hz, 1H), 4.19 (dt, J = 12.42, 4.02 Hz, 1H), 3.84 (t, J = 7.39 Hz, 2H), 3.07 (d, J = 11.59 Hz, 2H), 2.45 (t, J = 7.39 Hz, 2H), 2.38 (s, 3H), 2.33 (dq, J = 12.25, 3.52 Hz, 2H), 2.11 (t, J = 11.58 Hz, 2H), 1.95-1.84 (m, 4H), 1.63 (m, 2H).

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EXAMPLE 131

5 **5-Methylthio-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

10 ¹H NMR (400 MHz, CDCl₃) 7.78 (t, J = 1.68 Hz, 1H), 7.75 (s, 1H), 7.62 (dd, J = 8.23, 1.85 Hz, 1H), 7.15 (br d, J = 7.39 Hz, 1H), 7.02 (s, 1H), 6.94 (d, J = 8.06 Hz, 1H), 4.19 (m, 1H), 3.82 (t, J = 7.22 Hz, 2H), 3.08 (br d, J = 9.56 Hz, 2H), 2.60 (s, 3H), 2.46 (br m, 2H), 2.37 (s, 3H), 2.42-2.37 (br m, 2H), 2.15-2.11 (br m, 2H), 1.94-1.83 (br m, 4H), 1.66-1.64 (br m, 2H).

15

EXAMPLE 132

5-Ethoxy-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

20 ¹H NMR (400 MHz, CDCl₃) 7.79 (dd, J = 8.56, 3.53 Hz, 1H), 7.44 (dd, J = 4.36, 2.35 Hz, 1H), 7.29 (m, 1H), 7.14 (d, J = 8.06 Hz, 1H), 7.02 (s, 1H), 6.94 (br d, J = 7.22 Hz, 1H), 4.18 (m, 3H), 3.81 (t, J = 7.38 Hz, 2H), 3.71 (t, J = 6.38 Hz, 1H), 3.06 (d, J = 11.75 Hz, 2H), 2.44 (t, J = 7.38 Hz, 2H), 2.37 (s, 3H), 2.31 (dt, J = 12.26, 3.53 Hz, 1H), 2.10 (dt, J = 11.92, 2.02 Hz, 2H), 1.97-1.82 (m, 4H), 1.73-1.61 (m, 2H), 1.48 (dt, J = 7.06, 1.18 Hz, 3H).

25

EXAMPLE 133

30 **5-Chloro-1,1-dioxido-2-(4-(4-(6-fluoro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

mp 155-156 °C ¹H NMR (400 MHz, CDCl₃) 8.04 (dd, 1H), 7.89 - 7.82 (m, 2H), 7.18 (q, 1H), 7.00 (dd, 1H), 6.89 (td, 1H), 4.19 (m, 1H), 3.82

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(t, 2H), 3.07 (d, 2H), 2.45 (t, 2H), 2.40-2.27 (m, 2H), 2.11 (td, 2H), 1.97-1.82 (m, 4H), 1.64 (m, 2H).

EXAMPLE 134

5 **4-Methyl-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

mp 137-138 °C ¹H NMR (400 MHz, CDCl₃) 7.78-7.69 (m, 2H), 7.58 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 4.7 Hz, 1H), 7.20 (dd, J = 6.9 Hz, 1.4 Hz, 1H), 7.17-7.06 (m, 2H), 4.21 (m, 1H), 3.81 (t, J = 14.8 Hz, 2H), 3.07 (d, J = 11.6 Hz, 2H), 2.79 (s, 3H), 2.46 (t, J = 14.8 Hz, 2H), 2.41-2.31 (m, 2H), 2.12 (t, J = 23.8 Hz, 2H), 1.97-1.81 (m, 4H), 1.69-1.59 (m, 2H).

15

EXAMPLE 135

4-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

mp 178-180 °C ¹H NMR (400 MHz, CDCl₃) 7.88-7.84 (m, 1H), 7.82-7.76 (m, 2H), 7.31-7.26 (m, 1H), 7.24-7.08 (m, 3H), 4.21 (m, 1H), 3.84 (t, J = 14.6 Hz, 2H), 3.09 (d, J = 9.6 Hz, 2H), 2.46 (t, J = 14.5 Hz, 2H), 2.43-2.33 (m, 2H), 2.12 (t, J = 23.8 Hz, 2H), 1.99-1.83 (m, 4H), 1.71-1.59 (m, 2H).

25

EXAMPLE 136

4-Ethoxy-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

¹H NMR (400 MHz, CDCl₃) 7.77 (t, J = 7.6 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.28-7.27 (m, 2H), 7.19 (d, J = 7.6 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 7.08 (td, J = 7.6, 1.4 Hz, 1H), 4.28 (q, J = 7.0 Hz, 2H), 4.26 (tt, J = 12.6, 4.2 Hz, 1H), 3.78 (t, J = 7.4 Hz, 2H), 3.07 (d, J = 11.6 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.36 (td, J = 12.5, 3.7 Hz, 2H), 2.11 (td, J

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= 12.0, 1.6 Hz, 2H), 1.93-1.83 (m, 4H), 1.63 (qn, J = 7.6 Hz, 2H), 1.55 (t, J = 7.0 Hz, 3H).

EXAMPLE 137

5 **4-(2-Propyloxy)-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

10 ¹H NMR (400 MHz, CDCl₃) 7.75 (dd, J = 8.4, 7.7 Hz, 1H), 7.43 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.26 (d, J = 8.6 Hz, 1H), 7.19 (dd, J = 7.8, 1.2 Hz, 1H), 7.14 (td, J = 7.7, 1.3 Hz, 1H), 7.08 (td, J = 7.8, 1.2 Hz, 1H), 4.77 (sept., J = 6.0 Hz, 1H), 4.21 (tt, J = 12.5, 4.3 Hz, 1H), 3.77 (t, J = 7.4 Hz, 2H), 3.07 (d, J = 11.7 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 2.36 (td, J = 12.6, 3.8 Hz, 2H), 2.11 (td, J = 11.9, 1.8 Hz, 2H),
15 1.94-1.83 (m, 4H), 1.63 (qn, J = 7.5 Hz, 2H), 1.47 (d, J = 6.0 Hz, 6H).

EXAMPLE 138

20 **5-Methoxy-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 7.81 (d, J = 8.6 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 2.2 Hz, 1H), 7.33 (dd, J = 8.6, 2.4 Hz, 1H), 7.07 (d, J = 7.9 Hz, 1H), 7.03 (s, 1H), 4.56 (t, J = 12.6 Hz, 1H), 3.97 (s, 3H),
25 3.83 (t, J = 6.6 Hz, 2H), 3.72 (d, J = 11.0 Hz, 2H), 3.25 (m, 2H), 3.11 (m, 2H), 2.90 (m, 2H), 2.37 (s, 3H), 2.09-1.96 (m, 6H).

EXAMPLE 139

30 **1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-6-methylsulfonyl-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 8.51 (s, 1H), 8.40 (d, J = 9.57 Hz, 1H), 8.27 (d, J = 7.89 Hz, 1H), 7.20 (d, J = 7.73 Hz, 1H), 7.16-7.08 (m, 3H), 4.21 (m, 1H), 3.89 (t, J = 7.39 Hz, 2H), 3.17 (s, 3H), 3.12 (bd, 2H),

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2.40 (s, 2H), 2.37 (d, 2H), 1.93 (t, J = 7.73 Hz, 2H), 1.87 (bd, 2H), 1.60 (bm, 4H).

EXAMPLE 140

5 **5-Methoxy-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

¹H NMR (400 MHz, CDCl₃) 7.93 (d, J = 7.87 Hz, 1H), 7.82 (d, J = 8.60 Hz, 1H), 7.48 (d, J = 2.38 Hz, 1H), 7.34 (dd, J = 6.22, 2.38 Hz, 1H),
10 7.27 (m, 1H), 7.20 (d, J = 7.14, 1H), 7.13 (t, J = 7.69 Hz, 1H), 4.60 (tt, J = 12.81, 4.03 Hz, 1H), 3.97 (s, 3H), 3.83 (t, J = 6.59 Hz, 2H), 3.74 (d, J = 12.09 Hz, 2H), 3.32 (dq, J = 12.82, 4.03 Hz, 2H), 3.12 (br t, J = 4.58 Hz, 2H), 2.92 (br q, J = 10.07 Hz, 2H), 2.14-1.95 (m, 6H).

15

EXAMPLE 141

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-methylsulfonyl-1,2-benzisothiazol-3(2H)-one

20 ¹H NMR (400 MHz, DMSO-d₆) 8.67 (d, J = 8.06 Hz, 1H), 8.57 (dd, J = 8.06, 1.51 Hz, 1H), 8.54 (s, 1H), 7.39 (m, 2H), 7.26 (t, J = 7.88 Hz, 1H), 7.17 (t, J = 7.72 Hz, 1H), 4.49 (dt, J = 11.92, 4.01 Hz, 1H), 3.85 (bm, 2H), 3.64 (br d, J = 11.92 Hz, 2H), 3.44 (s, 3H), 3.20-3.10 (m, 2H), 2.54 (m, 2H), 2.08 (br d, J = 12.59 Hz, 2H).

25

EXAMPLE 142

5-Methylthio-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one

30 ¹H NMR (400 MHz, CDCl₃) 7.93 (d, J = 7.87 Hz, 1H), 7.77 (m, 2H), 7.63 (dd, J = 8.24, 1.83 Hz, 1H), 7.27 (m, 1H), 7.20 (dd, J = 8.05, 1.10 Hz, 1H), 7.13 (t, J = 8.42, 0.92 Hz, 1H), 4.60 (dt, J = 12.81, 4.22 Hz, 1H), 3.84 (t, J = 6.59 Hz, 2H), 3.74 (br d, J = 11.54 Hz, 2H), 3.33 (dq, J

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= 13.55, 3.84 Hz, 2H), 3.13 (m, 2H), 2.93 (q, J = 10.07 Hz, 2H), 2.60 (s, 3H), 2.14-1.93 (m, 6H).

EXAMPLE 143

5 **5-Methylsulfonyl-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

10 ¹H NMR (400 MHz, CDCl₃) 8.63 (s, 1H), 8.46 (dd, J = 8.05, 1.65 Hz, 1H), 8.15 (dd, J = 8.05, 0.55 Hz, 1H), 7.13 (d, J = 8.06 Hz, 1H), 7.03 (s, 1H), 6.95 (d, J = 8.05 Hz, 1H), 4.18 (dt, J = 12.01, 4.21 Hz, 1H), 3.89 (t, J = 7.33 Hz, 2H), 3.16 (s, 3H), 3.07 (br d, J = 10.99 Hz, 2H), 2.46 (t, J = 7.14 Hz, 2H), 2.38 (s, 3H), 2.34 (m, 2H), 2.11 (t, J = 11.17 Hz, 2H), 1.97-1.84 (m, 6H).

EXAMPLE 144

20 **1,1-Dioxido-2-(4-(4-(2-oxo-1-oxazolo[5,4-b]pyridyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one**

25 ¹H NMR (400 MHz, CDCl₃) 8.38 (d, J = 7.51 Hz, 1H), 8.07 (m, 2H), 7.91 (m, 3H), 7.25 (m, 1H), 4.64 (m, 1H), 3.86 (t, J = 3.20 Hz, 2H), 3.74 (br d, J = 10.99 Hz, 2H), 3.29 (m, 2H), 3.13 (bs, 2H), 2.92 (m, 2H), 2.18-1.99 (m, 6H).

30

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Thompson, Wayne J.
Huff, Joel R.
Nerenberg, Jennie B.
Lee, Hee-Yoon
Bell, Ian M.

(ii) TITLE OF INVENTION: ALPHA1C ADRENERGIC RECEPTOR ANTAGONISTS

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Merck & Co., Inc.
(B) STREET: 126 Lincoln Avenue
(C) CITY: Rahway
(D) STATE: New Jersey
(E) COUNTRY: United States of America
(F) ZIP: 07065

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/229,276
(B) FILING DATE: 14-APR-1995

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Appollina, Mary A.
(B) REGISTRATION NUMBER: 34,087
(C) REFERENCE/DOCKET NUMBER: 19169Y

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (908)594-3462
(B) TELEFAX: (908)594-4720
(C) TELEX: 138825

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTTTCTAGAT TRTTNARRTA NCCNAGCC

28

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTTACTAGTA TCSTNGTNAT GTAYTG

26

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTTCTAGAG AARAANGGNA RCCARC

26

(2) INFORMATION FOR SEQ ID NO:4:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 235 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

GCCGCGTCTA CGTGGTGGCC AAGAGGGAGA GCCGGGGCCT CAAGTCTGGC CTCAAGACCG      60
ACAAGTCGGA CTCGGAGCAA GTGACGCTCC GCATCCATCG GAAAAACGCC CCGGCAGGAG      120
GCAGCGGGAT GGCCAGCGCC AAGACCAAGA CGCACTTCTC AGTGAGGCTC CTCAAGTTCT      180
CCCGGGAGAA GAAAGCGGCC AAAACGCTGG GCATCGTGGT CGGCTGCTTC GTCCT          235
  
```

- (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 78 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (v) FRAGMENT TYPE: internal

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Arg Val Tyr Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly
 1           5           10           15
Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile His
 20           25           30
Arg Lys Asn Ala Pro Ala Gly Gly Ser Gly Met Ala Ser Ala Lys Thr
 35           40           45
Lys Thr His Phe Ser Val Arg Leu Leu Lys Phe Ser Arg Glu Lys Lys
 50           55           60
  
```

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Ala Ala Lys Thr Leu Gly Ile Val Val Gly Cys Phe Val Leu
 65 70 75

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 93 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Val Met Tyr Cys Arg Val Tyr Val Val Ala Lys Arg Glu Ser Arg
 1 5 10 15
 Gly Leu Lys Ser Gly Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln Val
 20 25 30
 Thr Leu Arg Ile His Arg Lys Asn Ala Gln Val Gly Gly Ser Gly Val
 35 40 45
 Thr Ser Ala Lys Asn Lys Thr His Phe Ser Val Arg Leu Leu Lys Phe
 50 55 60
 Ser Arg Glu Lys Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly Cys
 65 70 75 80
 Phe Val Leu Cys Trp Leu Pro Phe Phe Leu Val Met Pro
 85 90

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1601 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAATTCCCTC CTAGAAGCTG GAGAGAGCAG GAGCCTTCGG TGGGGCAGCT CAAAATGTAG	60
GTAAC TGCGG GCCAGGAGCA GCGCCAGAT GCCATCGGTC CCTGCCTTTG AGCGTCGACG	120
GCTGATCTTT TGGTTTGAGG GAGAGACTGG CGCTGGAGTT TTGAATTCCG AATCATGTGC	180
AGAATCGTGA ATCTTCCCCC AGCCAGGACG AATAAGACAG CGCGGAAAAG CAGATTCTCG	240
TAATTCTGGA ATTGCATGTT GCAAGGAGTC TCCTGGATCT TCGCACCCAG CTCGGGTAC	300
GGGAGGGAGT CCGGGTCCCG GCTAGGCCAG CCCGCAGGTG GAGAGGGTCC CCGGCAGCCC	360
CGCGCGCCCC TGGCCATGTC TTTAATGCCC TGCCCTTCA TGTGGCCTTC TGAGGGTTCC	420
CAGGGCTGGC CAGGGTTGTC TCCCACCCGC GCGCGCCGTC TCACCCCCAG CCAAACCCAC	480
CTGGCAGGGC TCCCTCCAGA AGAGACCTTT TGATTCCCGG CTCCCGCGCT CCCGCTCCG	540
CGCCAGCCCC GGAGTGCGC CTGGACAGCC GGACCTCGCC CGGCCCGGC TGGGACCATG	600
GTGTTTCTCT CGGGAAATGC TTCCGACAGC TCCAAC TGCA CCCAACCGCC GGCACCGGTG	660
AACATTTCCA AGGCCATTCT GCTCGGGGTG ATCTTGGGGG GCCTCATTCT TTTCGGGGTG	720
CTGGGTAAACA TCCTAGTGAT CCTCTCCGTA GCCTGTCACC GACACCTGCA CTCAGTCACG	780
CACTACTACA TCGTCAACCT GGCGGTGGCC GACCTCCTGC TCACCTCCAC GGTGCTGCCC	840
TTCTCCGCCA TCTTCGAGGT CCTAGGCTAC TGGGCCTTCG GCAGGGTCTT CTGCAACATC	900
TGGGCGGCAG TGGATGTGCT GTGCTGCACC GCGTCCATCA TGGGCCTCTG CATCATCTCC	960
ATCGACCGCT ACATCGGCGT GAGCTACCCG CTGCGCTACC CAACCATCGT CACCCAGAGG	1020
AGGGGTCTCA TGGCTCTGCT CTGCGTCTGG GCACTCTCCC TGGTCATATC CATTGGACCC	1080
CTCTTCGGCT GGAGGCAGCC GGCCCCGAG GACGAGACCA TCTGCCAGAT CAACGAGGAG	1140
CCGGGCTACG TGCTCTTCTC GGCTCTGGGC TCCTTCTACC TGCCTCTGGC CATCATCTCTG	1200
GTCAATGTACT GCCGCGTCTA CGTGGTGGCC AAGAGGGAGA GCCGGGGCCT CAAGTCTGGC	1260
CTCAAGACCG ACAAGTCGGA CTCGGAGCAA GTGACGCTCC GCATCCATCG GAAAAACGCC	1320
CCGGCAGGAG GCAGCGGGAT GGCCAGCGCC AAGACCAAGA CGCACTTCTC AGTGAGGCTC	1380
CTCAAGTTCT CCCGGGAGAA GAAAGCGGCC AAAACGCTGG GCATCGTGGT CGGCTGCTTC	1440
GTCTCTGCT GGCTGCCTTT TTTCTTAGTC ATGCCCATG GGTCTTTCTT CCCTGATTTT	1500
AAGCCCTCTG AAACAGTTTT TAAAATAGTA TTTTGGCTCG GATATCTAAA CAGCTGCATC	1560
AACCCCATCA TATACCATG CTCCAGCCAA GAGGGAATTC C	1601

(2) INFORMATION FOR SEQ ID NO:8:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTGAATTCT GATTCAAGC CCTCTG

26

- (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTGAATTCT TANACYTCYT CNCCRTTYTC

30

- (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 512 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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```

CTGATTTCAA GCCCTCTGAA ACAGTTTTTA AAATAGTATT TTGGCTCGGA TATCTAAACA      60
GCTGCATCAA CCCCATCATA TACCCATGCT CCAGCCAAGA GTTCAAAAAG GCCTTTCAGA      120
ATGTCTTGAG AATCCAGTGT CTCCGCAGAA AGCAGTCTTC CAAACATGCC CTGGGCTACA      180
CCCTGCACCC GCCCAGCCAG GCCGTGGAAG GGCAACACAA GGACATGGTG CGCATCCCCG      240
TGGGATCAAG AGAGACCTTC TACAGGATCT CCAAGACGGA TGGCGTTTGT GAATGGAAAT      300
TTTTCTCTTC CATGCCCCGT GGATCTGCCA GGATTACAGT GTCCAAAGAC CAATCCTCCT      360
GTACCACAGC CCGGGTGAGA AGTAAAAGCT TTTTGCAGGT CTGCTGCTGT GTAGGGCCCT      420
CAACCCCCAG CCTTGACAAG AACCATCAAG TTCCAACCAT TAAGGTCCAC ACCATCTCCC      480
TCAGTGAGAA CGGCGAAGAG GTTTAAGAAT TC                                     512

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2004 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

GAATTCCCTC CTAGAAGCTG GAGAGAGCAG GAGCCTTCGG TGGGGCAGCT CAAAATGTAG      60
GTAAC TGCGG GCCAGGAGCA GCGCCCAGAT GCCATCGGTC CCTGCCTTTG AGCGTCGACG      120
GCTGATCTTT TGGTTTGAGG GAGAGACTGG CGCTGGAGTT TTGAATTCCG AATCATGTGC      180
AGAATCGTGA ATCTTCCCCC AGCCAGGACG AATAAGACAG CGCGAAAAG CAGATTCTCG      240
TAATTCTGGA ATTGCATGTT GCAAGGAGTC TCCTGGATCT TCGCACCCAG CTTCGGGTAC      300
GGGAGGGGAGT CCGGGTCCCG GCTAGGCCAG CCCGCAGGTG GAGAGGGTCC CCGGCAGCCC      360
CGCGCGCCCC TGGCCATGTC TTTAATGCCC TGCCCCTTCA TGTGGCCTTC TGAGGGTTCC      420
CAGGGCTGGC CAGGGTTGTC TCCCACCCGC GCGCGCCGTC TCACCCCCAG CCAAACCCAC      480
CTGGCAGGGC TCCCTCCAGA AGAGACCTTT TGATTCCCGG CTCCCGCGCT CCCGCCTCCG      540
CGCCAGCCCG GGAGGTGGCC CTGGACAGCC GGACCTCGCC CGGCCCCGGC TGGGACCATG      600

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GTGTTTCTCT CGGGAAATGC TTCCGACAGC TCCAAC TGCA CCCAACCGCC GGCACCGGTG	660
AACATTTCCA AGGCCATTCT GCTCGGGGTG ATCTTGGGGG GCCTCATTCT TTTGGGGGTG	720
CTGGGTAACA TCCTAGTGAT CCTCTCCGTA GCCTGTCACC GACACCTGCA CTCAGTCACG	780
CACTACTACA TCGTCAACCT GGCAGTGGCC GACCTCCTGC TCACCTCCAC GGTGCTGCCC	840
TTCTCCGCCA TCTTCGAGGT CCTAGGCTAC TGGGCCTTCG GCAGGGTCTT CTGCAACATC	900
TGGGCGGCAG TGGATGTGCT GTGCTGCACC GCGTCCATCA TGGGCCTCTG CATCATCTCC	960
ATCGACCGCT ACATCGGCGT GAGCTACCCG CTGCGCTACC CAACCATCGT CACCCAGAGG	1020
AGGGGTCTCA TGGCTCTGCT CTGCGTCTGG GCACTCTCCC TGGTCATATC CATTGGACCC	1080
CTCTTCGGCT GGAGGCAGCC GGCCCCGAG GACGAGACCA TCTGCCAGAT CAACGAGGAG	1140
CCGGGCTACG TGCTCTTCTC GGCTCTGGGC TCCTTCTACC TGCCTCTGGC CATCATCCTG	1200
GTCATGTACT GCCGCGTCTA CGTGGTGGCC AAGAGGGAGA GCCGGGGCCT CAAGTCTGGC	1260
CTCAAGACCG ACAAGTCGGA CTCGGAGCAA GTGACGCTCC GCATCCATCG GAAAAACGCC	1320
CCGGCAGGAG GCAGCGGGAT GGCCAGCGCC AAGACCAAGA CGCACTTCTC AGTGAGGCTC	1380
CTCAAGTTCT CCCGGGAGAA GAAAGCGGCC AAAACGCTGG GCATCGTGGT CGGCTGCTTC	1440
GTCCTCTGCT GGCTGCCTTT TTTCTTAGTC ATGCCCATTG GGTCTTTCTT CCCTGATTTC	1500
AAGCCCTCTG AAACAGTTTT TAAAATAGTA TTTTGGCTCG GATATCTAAA CAGCTGCATC	1560
AACCCCATCA TATACCCATG CTCCAGCCAA GAGTTCAAAA AGGCCTTTCA GAATGTCTTG	1620
AGAATCCAGT GTCTCCGCGAG AAAGCAGTCT TCCAAACATG CCCTGGGCTA CACCCTGCAC	1680
CCGCCCAGCC AGGCCGTGGA AGGGCAACAC AAGGACATGG TGCGCATCCC CGTGGGATCA	1740
AGAGAGACCT TCTACAGGAT CTCCAAGACG GATGGCGTTT GTGAATGGAA ATTTTCTCT	1800
TCCATGCCCC GTGGATCTGC CAGGATTACA GTGTCCAAAG ACCAATCCTC CTGTACCACA	1860
GCCCGGGTGA GAAGTAAAAG CTTTTTGACG GTCTGCTGCT GTGTAGGGCC CTCAACCCCC	1920
AGCCTTGACA AGAACCATCA AGTTCCAACC ATTAAGGTCC ACACCATCTC CCTCAGTGAG	1980
AACGGCGAAG AGGTTTAAGA ATTC	2004

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 466 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Val Phe Leu Ser Gly Asn Ala Ser Asp Ser Ser Asn Cys Thr Gln
 1             5             10             15

Pro Pro Ala Pro Val Asn Ile Ser Lys Ala Ile Leu Leu Gly Val Ile
      20             25             30

Leu Gly Gly Leu Ile Leu Phe Gly Val Leu Gly Asn Ile Leu Val Ile
   35             40             45

Leu Ser Val Ala Cys His Arg His Leu His Ser Val Thr His Tyr Tyr
 50             55             60

Ile Val Asn Leu Ala Val Ala Asp Leu Leu Leu Thr Ser Thr Val Leu
 65             70             75             80

Pro Phe Ser Ala Ile Phe Glu Val Leu Gly Tyr Trp Ala Phe Gly Arg
      85             90             95

Val Phe Cys Asn Ile Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala
    100             105             110

Ser Ile Met Gly Leu Cys Ile Ile Ser Ile Asp Arg Tyr Ile Gly Val
   115             120             125

Ser Tyr Pro Leu Arg Tyr Pro Thr Ile Val Thr Gln Arg Arg Gly Leu
    130             135             140

Met Ala Leu Leu Cys Val Trp Ala Leu Ser Leu Val Ile Ser Ile Gly
    145             150             155             160

Pro Leu Phe Gly Trp Arg Gln Pro Ala Pro Glu Asp Glu Thr Ile Cys
    165             170             175

Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu Gly Ser
    180             185             190

Phe Tyr Leu Pro Leu Ala Ile Ile Leu Val Met Tyr Cys Arg Val Tyr
    195             200             205

Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly Leu Lys Thr
    210             215             220

Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile His Arg Lys Asn
    225             230             235             240

```


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[illegible]

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1621 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCCGTGCAGG GGCCCTACGG ACACCACCAG GGCTACGACC CAGAGCAGGG CCAGGATGGC	60
GGCCGCCTTG CGCTCGGTCA TGATGGCTGG GTACTTGAGT GAGTGGCGCA CGCCCACGTA	120
CCGGTCCACG GAGATGGTGC AGAGGCTGAG GATGGAGGCC GTGCAGCACA GCACGTCCAC	180
GGCGGCCGTC GGGGGACTGG TGGTGAGCGC GCAGGGCGTG GGCGTGGGCG TCTTCCTGGC	240
AGCCTTCATC CTTATGGCCG TGGCAGGTAA CCTGCTTGTC ATCCTCTCAG TGGCCTGCAA	300
CCGCCACCTG CAGACCGTCA CCAACTATTT CATCGTGAAC CTGGCCGTGG CCGACCTGCT	360
GCTGAGCGCC ACCGTACTGC CCTTCTCGGC CACCATGGAG GTTCTGGGCT TCTGGGCCCTT	420
TGGCCGCGCC TTCTGCGACG TATGGGCCCG CGTGACGTG CTGTGCTGCA CGGCCTCCAT	480
CCTCAGCCTC TGCACCATCT CCGTGGACCG GTACGTGGGC GTGCGCCACT CACTCAAGTA	540
CCCAGCCATC ATGACCGAGC GCAAGGCGGC CGCCATCCTG GCCCTGCTCT GGGTCGTAGC	600
CCTGGTGGTG TCCGTAGGGC CCCTGCTGGG CTGGAAGGAG CCCGTGCCCC CTGACGAGCG	660
CTTCTGCGGT ATCACCGAGG AGGCGGGCTA CGCTGTCTTC TCCTCCGTGT GCTCCTTCTA	720
CCTGCCCCATG GCGGTCATCG TGGTCATGTA CTGCCGCGTG TACGTGGTCG CGCGCAGCAC	780
CACGCGCAGC CTCGAGGAGC GCGTCAAGCG CGAGCGAGGC AAGGCCTCCG AGGTGGTGCT	840
GCGCATCCAC TGTCGCGGCG CGGCCACGGG CGCCGACGGG GCGCACGGCA TGCGCAGCGC	900
CAAGGGCCAC ACCTTCCGCA GCTCGCTCTC CGTGCGCCTG CTCAAGTTCT CCCGTGAGAA	960
GAAAGCGGCC AAGACTCTGG CCATCGTCGT GGGTGTCTTC GTGCTCTGCT GGTTCCTTTT	1020
CTTCTTTGTC CTGCCGCTCG GCTCCTTGTT CCCGAGCTG AAGCCATCGG AGGGCGTCTT	1080
CAAGGTCATC TTCTGGCTCG GCTACTTCAA CAGCTGCGTG AACCCGCTCA TCTACCCCTG	1140
TTCCAGCCGC GAGTTCAAGC GCGCCTTCCT CCGTCTCCTG CGCTGCCAGT GCCGTCGTCG	1200
CCGGCGCCGC CGCCCTCTCT GGCCTGTCTA CGGCCACCAC TGGCGGGCCT CCACCAGCGG	1260
CCTGCGCCAG GACTGCGCCC CGAGTTCGGG CGACGCGCCC CCCGAGCGC CGCTGGCCCT	1320
CACCGCGCTC CCCGACCCCG ACCCCGAACC CCCAGGCACG CCCGAGATGC AGGCTCCGGT	1380
CGCCAGCCGT CGAAGCCACC CAGCGCCTTC CGCGAGTGGA GGCTGCTGGG GCCGTTCGGG	1440
AGACCCACGA CCCAGCTGCG CGCCAAAGTC GCCAGCCTGT CGCACAAGAT CGCCGCCGGG	1500

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GGCGCGCAGC GCGCAGAGGC AGCGTGCGCC CAGCGCTCAG AGGTGGAGGC TGTGTCCCTA 1560
 GGCGTCCCAC ACGAGGTGGC CGAGGGCGCC ACCTGCCAGG CCTACGAATT GGCCGACTAC 1620
 A 1621

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 501 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met	Ala	Ala	Ala	Leu	Arg	Ser	Val	Met	Met	Ala	Gly	Tyr	Leu	Ser	Glu	1	5	10	15
Trp	Arg	Thr	Pro	Thr	Tyr	Arg	Ser	Thr	Glu	Met	Val	Gln	Arg	Leu	Arg	20	25	30	
Met	Glu	Ala	Val	Gln	His	Ser	Thr	Ser	Thr	Ala	Ala	Val	Gly	Gly	Leu	35	40	45	
Val	Val	Ser	Ala	Gln	Gly	Val	Gly	Val	Gly	Val	Phe	Leu	Ala	Ala	Phe	50	55	60	
Ile	Leu	Met	Ala	Val	Ala	Gly	Asn	Leu	Leu	Val	Ile	Leu	Ser	Val	Ala	65	70	75	80
Cys	Asn	Arg	His	Leu	Gln	Thr	Val	Thr	Asn	Tyr	Phe	Ile	Val	Asn	Leu	85	90	95	
Ala	Val	Ala	Asp	Leu	Leu	Leu	Ser	Ala	Thr	Val	Leu	Pro	Phe	Ser	Ala	100	105	110	
Thr	Met	Glu	Val	Leu	Gly	Phe	Trp	Ala	Phe	Gly	Arg	Ala	Phe	Cys	Asp	115	120	125	

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Val	Trp	Ala	Ala	Val	Asp	Val	Leu	Cys	Cys	Thr	Ala	Ser	Ile	Leu	Ser	
130						135					140					
Leu	Cys	Thr	Ile	Ser	Val	Asp	Arg	Tyr	Val	Gly	Val	Arg	His	Ser	Leu	
145					150					155					160	
Lys	Tyr	Pro	Ala	Ile	Met	Thr	Glu	Arg	Lys	Ala	Ala	Ala	Ile	Leu	Ala	
				165					170					175		
Leu	Leu	Trp	Val	Val	Ala	Leu	Val	Val	Ser	Val	Gly	Pro	Leu	Leu	Gly	
			180					185					190			
Trp	Lys	Glu	Pro	Val	Pro	Pro	Asp	Glu	Arg	Phe	Cys	Gly	Ile	Thr	Glu	
		195					200					205				
Glu	Ala	Gly	Tyr	Ala	Val	Phe	Ser	Ser	Val	Cys	Ser	Phe	Tyr	Leu	Pro	
210						215					220					
Met	Ala	Val	Ile	Val	Val	Met	Tyr	Cys	Arg	Val	Tyr	Val	Val	Ala	Arg	
225					230					235					240	
Ser	Thr	Thr	Arg	Ser	Leu	Glu	Ala	Gly	Val	Lys	Arg	Glu	Arg	Gly	Lys	
				245					250					255		
Ala	Ser	Glu	Val	Val	Leu	Arg	Ile	His	Cys	Arg	Gly	Ala	Ala	Thr	Gly	
			260					265					270			
Ala	Asp	Gly	Ala	His	Gly	Met	Arg	Ser	Ala	Lys	Gly	His	Thr	Phe	Arg	
		275					280					285				
Ser	Ser	Leu	Ser	Val	Arg	Leu	Leu	Lys	Phe	Ser	Arg	Glu	Lys	Lys	Ala	
		290				295					300					
Ala	Lys	Thr	Leu	Ala	Ile	Val	Val	Gly	Val	Phe	Val	Leu	Cys	Trp	Phe	
305					310					315					320	
Pro	Phe	Phe	Phe	Val	Leu	Pro	Leu	Gly	Ser	Leu	Phe	Pro	Gln	Leu	Lys	
				325					330					335		
Pro	Ser	Glu	Gly	Val	Phe	Lys	Val	Ile	Phe	Trp	Leu	Gly	Tyr	Phe	Asn	
			340					345					350			
Ser	Cys	Val	Asn	Pro	Leu	Ile	Tyr	Pro	Cys	Ser	Ser	Arg	Glu	Phe	Lys	
		355					360					365				
Arg	Ala	Phe	Leu	Arg	Leu	Leu	Arg	Cys	Gln	Cys	Arg	Arg	Arg	Arg	Arg	
					375						380					
Arg	Arg	Pro	Leu	Trp	Arg	Val	Tyr	Gly	His	His	Trp	Arg	Ala	Ser	Thr	
385					390					395					400	
Ser	Gly	Leu	Arg	Gln	Asp	Cys	Ala	Pro	Ser	Ser	Gly	Asp	Ala	Pro	Pro	
				405					410					415		
Gly	Ala	Pro	Leu	Ala	Leu	Thr	Ala	Leu	Pro	Asp	Pro	Asp	Pro	Glu	Pro	
			420					425						430		

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Pro Gly Thr Pro Glu Met Gln Ala Pro Val Ala Ser Arg Arg Ser His
 435 440 445

Pro Ala Pro Ser Ala Ser Gly Gly Cys Trp Gly Arg Ser Gly Asp Pro
 450 455 460

Arg Pro Ser Cys Ala Pro Lys Ser Pro Ala Cys Arg Thr Arg Ser Pro
 465 470 475 480

Pro Gly Ala Arg Ser Ala Gln Arg Gln Arg Ala Pro Ser Ala Gln Arg
 485 490 495

Trp Arg Leu Cys Pro
 500

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCTAGACCAT GAAYCCNGAY CTGG

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(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTTGAATTCA CATWCCGACY ACAATGCCC

29

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(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 921 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCTAGACCAT GAATCCCGAC CTGGACACCG GCCACAACAC ATCAGCACCT GCCCACTGGG	60
GAGAGTTGAA AAATGCCAAC TTCACTGGCC CCAACCAGAC CTCGAGCAAC TCCACACTGC	120
CCCAGCTGGA CATCACCAGG GCCATCTCTG TGGGCCTGGT GCTGGGCGCC TTCATCCTCT	180
TTGCCATCGT GGGCAACATC CTAGTCATCT TGTCTGTGGC CTGCAACCGG CACCTGCGGA	240
CGCCACCAA CTA CTTCATT GTCAACCTGG CCATGGCCGA CCTGCTGTTG AGCTTCACCG	300
TCCTGCCCTT CTCAGCGGCC CTAGAGGTGC TCGGCTACTG GGTGCTGGGG CGGATCTTCT	360
GTGACATCTG GGCAGCCGTG GATGTCCTGT GCTGCACAGC GTCCATTCTG AGCCTGTGCG	420
CCATCTCCAT CGATCGCTAC ATCGGGGTGC GCTACTCTCT GCAGTATCCC ACGCTGGTCA	480
CCCGGAGGAA GGCCATCTTG GCCCTGCTCA GTGTCTGGGT CTGTGCCACC GTCATCTCCA	540
TCGGGCCTCT CCTTGGGTGG AAGGAGCCGG CACCCAACGA TGACAAGGAG TCGGGGGTCA	600
CCGAAGAACC CTTCTATGCC CTCTTCTCCT CTCTGGGCTC CTTCTACATC CCTCTGGCGG	660
TCATTCTAGT CATGTACTGC CGTGTCTATA TAGTGGCCAA GAGAACCACC AAGAACCTAG	720
AGGCAGGAGT CATGAAGGAG ATGTCCAACCT CCAAGGAGCT GACCCTGAGG ATCCATTCCA	780
AGAACTTTCA CGAGGACACC CTTAGCAGTA CCAAGGCCAA GGGCCACAAC CCCAGGAGTT	840
CCATAGCTGT CAAACTTTTT AAGTTCTCCA GGGAAAAGAA AGCAGCTAAG ACGTTGGGCA	900
TTGTGGTCGG TATGTGAATT C	921

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAAGGCGCGC TTGAACTC

18

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AGAGAACCAC CAAGAACC

18

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 389 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AAGAGAACCA CCAAGAACCT AGAGGCAGGA GTCATGAAGG AGATGTCCAA CTCCAAGGAG	60
CTGACCCTGA GGATCCATTC CAAGAACTTT CACGAGGACA CCCTTAGCAG TACCAAGGCC	120
AAGGGCCACA ACCCCAGGAG TTCCATAGCT GTCAAACCTT TTAAGTTCTC CAGGGAAAAG	180

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AAAGCAGCTA AGACGTTGGG CATTGTGGTC GGTATGTTCA TCTGTGCTG GCTACCCCTTC 240
TTCATCGCTC TACCGCTTGG CTCCTTGTTT TCCACCCTGA AGCCCCCGA CGCCGTGTTC 300
AAGGTGGTGT TCTGGCTGGG CTAATTCAAC AGCTGCCTCA ACCCCATCAT CTACCCATGC 360
TCCAGCAAGG AGTTCAAGCG CGCTTTCGT 389

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTTGAATTCA TGTTCAGGT GGTGTTC 27

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TTTGAATTCT AAAASTGNCC NGGNSCCAGN GGCAT 35

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 582 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GAATTCATGA TTCAAGGTGG TGTTCTGGCT GGGCTACTTC AACAGCTGCC TCAATCCCAT	60
CATCTACCCG TGCTCCAGCA AGGAGTTCAA GCGCGCCTTC ATGCGTATCC TTGGGTGCCA	120
TGCCCCGGGT GGCCGCCGCC GCCGCCGCCG TCGCCGTCTA GGC CGTGCG CTTACACCTA	180
CCGGCCGTGG ACCCGCGGCG GCTCGCTGGA GAGATCACAG TCGCGGAAGG ACTCTCTGGA	240
TGACAGCGGC AGCTGCATGA GCGGCCAGAA GAGGACCCTG CCCTCGGCGT CGCCAGCCCC	300
GGGCTACCTG GGTCGAGGAA CGCAGCCACC CGTGGAGCTG TCGCCTTCC CCGAGTGGA	360
ACCCGGGGCG CTGCTCAGCT TGCCAGAGCC TCCTGGCCGC CGCGGCCGTC TCGACTCTGG	420
GCCACTCTTC ACCTTCAAGC TCCTGGGCGA TCCTGAGAGC CCGGGAACCG AAGCGACAGC	480
CAGCAACGGG GGCTGCGACA CCACGACCGA CCTGGCCAAC GGCAGCCCG GCTTCAAGAG	540
CAACATGCCC CTGGGCCCGG GCCACTTTTA AAAGCCGAAT TC	582

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1567 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCTAGACCAT GAATCCCGAC CTGGACACCG GCCACAACAC ATCAGCACCT GCCCACTGGG	60
GAGAGTTGAA AAATGCCAAC TTTACTGGCC CCAACCAGAC CTCGAGCAAC TCCCACTGCG	120
CCCAGCTGGA CATCACCAGG GCCATCTCTG TGGGCCTGGT GCTGGGCGCC TTCATCCTCT	180
TTGCCATCGT GGGCAACATC CTAGTCATCT TGTCTGTGGC CTGCAACCGG CACCTGCGGA	240
CGCCCAACAA CTACTTCATT GTCAACCTGG CCATGGCCGA CCTGCTGTTG AGCTTCACCG	300

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TCCTGCCCTT CTCAGCGGCC CTAGAGGTGC TCGGCTACTG GGTGCTGGGG CGGATCTTCT	360
GTGACATCTG GGCAGCCGTG GATGTCTGT GCTGCACAGC GTCCATTCTG AGCCTGTGCG	420
CCATCTCCAT CGATCGCTAC ATCGGGGTGC GCTACTCTCT GCAGTATCCC ACGCTGGTCA	480
CCCGGAGGAA GGCCATCTTG GCCCTGCTCA GTGTCTGGGT CTTGTCCACC GTCATCTCCA	540
TCGGGCCTCT CCTTGGGTGG AAGGAGCCGG CACCCAACGA TGACAAGGAG TCGGGGTCA	600
CCGAAGAACC CTTCTATGCC CTCTTCTCCT CTCTGGGCTC CTTCTACATC CCTCTGGCGG	660
TCATTCTAGT CATGTACTGC CGTGTCTATA TAGTGGCCAA GAGAACCACC AAGAACCTAG	720
AGGCAGGAGT CATGAAGGAG ATGTCCAAC CCAAGGAGCT GACCCTGAGG ATCCATTCCA	780
AGAACTTTCA CGAGGACACC CTTAGCAGTA CCAAGGCCAA GGGCCACAAC CCCAGGAGTT	840
CCATAGCTGT CAAACTTTTT AAGTTCTCCA GGGAAAAGAA AGCAGCTAAG ACGTTGGGCA	900
TTGTGGTCGG TATGTTTCATC TTGTGCTGGC TACCCTTCTT CATCGCTCTA CCGCTTGGCT	960
CCTGTGTTCTC CACCCTGAAG CCCCCGACG CCGTGTTCAA GGTGGTGTTC TGGCTGGGCT	1020
ACTTCAACAG CTGCCTCAAC CCCATCATCT ACCCATGCTC CAGCAAGGAG TTCAAGCGCG	1080
CCTTCATGCG TATCCTTGGG TGCCAGTGCC GCGGTGGCCG CCGCCGCCGC CGCCGTCGCC	1140
GTCTAGGCGC GTGCGCTTAC ACCTACCGGC CGTGGACCCG CGGCGGCTCG CTGGAGAGAT	1200
CACAGTCGCG GAAGGACTCT CTGGATGACA GCGGCAGCTG CATGAGCGGC CAGAAGAGGA	1260
CCCTGCCCTC GGCCTCGCCC AGCCCGGGCT ACCTGGGTCG AGGAACGCAG CCACCCGTGG	1320
AGCTGTGCGC CTTCCCCGAG TGGAACCCG GGGCGCTGCT CAGCTTGCCA GAGCCTCCTG	1380
GCCGCCGCGG CCGTCTCGAC TCTGGGCCAC TCTTCACCTT CAAGCTCCTG GGCGATCCTG	1440
AGAGCCCGGG AACC GAAGCG ACAGCCAGCA ACGGGGGCTG CGACACCAG ACCGACCTGG	1500
CCAACGGGCA GCGCGGCTTC AAGAGCAACA TGCCCCTGGG CCGGGGCCAC TTTTAAAAGC	1560
CGAATTC	1567

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 515 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met	Asn	Pro	Asp	Leu	Asp	Thr	Gly	His	Asn	Thr	Ser	Ala	Pro	Ala	His	1	5	10	15
Trp	Gly	Glu	Leu	Lys	Asn	Ala	Asn	Phe	Thr	Gly	Pro	Asn	Gln	Thr	Ser	20	25	30	
Ser	Asn	Ser	Thr	Leu	Pro	Gln	Leu	Asp	Ile	Thr	Arg	Ala	Ile	Ser	Val	35	40	45	
Gly	Leu	Val	Leu	Gly	Ala	Phe	Ile	Leu	Phe	Ala	Ile	Val	Gly	Asn	Ile	50	55	60	
Leu	Val	Ile	Leu	Ser	Val	Ala	Cys	Asn	Arg	His	Leu	Arg	Thr	Pro	Thr	65	70	75	80
Asn	Tyr	Phe	Ile	Val	Asn	Leu	Ala	Met	Ala	Asp	Leu	Leu	Leu	Ser	Phe	85	90	95	
Thr	Val	Leu	Pro	Phe	Ser	Ala	Ala	Leu	Glu	Val	Leu	Gly	Tyr	Trp	Val	100	105	110	
Leu	Gly	Arg	Ile	Phe	Cys	Asp	Ile	Trp	Ala	Ala	Val	Asp	Val	Leu	Cys	115	120	125	
Cys	Thr	Ala	Ser	Ile	Leu	Ser	Leu	Cys	Ala	Ile	Ser	Ile	Asp	Arg	Tyr	130	135	140	
Ile	Gly	Val	Arg	Tyr	Ser	Leu	Gln	Tyr	Pro	Thr	Leu	Val	Thr	Arg	Arg	145	150	155	160
Lys	Ala	Ile	Leu	Ala	Leu	Leu	Ser	Val	Trp	Val	Leu	Ser	Thr	Val	Ile	165	170	175	
Ser	Ile	Gly	Pro	Leu	Leu	Gly	Trp	Lys	Glu	Pro	Ala	Pro	Asn	Asp	Asp	180	185	190	
Lys	Glu	Cys	Gly	Val	Thr	Glu	Glu	Pro	Phe	Tyr	Ala	Leu	Phe	Ser	Ser	195	200	205	
Leu	Gly	Ser	Phe	Tyr	Ile	Pro	Leu	Ala	Val	Ile	Leu	Val	Met	Tyr	Cys	210	215	220	
Arg	Val	Tyr	Ile	Val	Ala	Lys	Arg	Thr	Thr	Lys	Asn	Leu	Glu	Ala	Gly	225	230	235	240
Val	Met	Lys	Glu	Met	Ser	Asn	Ser	Lys	Glu	Leu	Thr	Leu	Arg	Ile	His	245	250	255	

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Ser Lys Asn Phe His Glu Asp Thr Leu Ser Ser Thr Lys Ala Lys Gly
 260 265 270
 His Asn Pro Arg Ser Ser Ile Ala Val Lys Leu Phe Lys Phe Ser Arg
 275 280 285
 Glu Lys Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly Met Phe Ile
 290 295 300
 Leu Cys Trp Leu Pro Phe Phe Ile Ala Leu Pro Leu Gly Ser Leu Phe
 305 310 315 320
 Ser Thr Leu Lys Pro Pro Asp Ala Val Phe Lys Val Val Phe Trp Leu
 325 330 335
 Gly Tyr Phe Asn Ser Cys Leu Asn Pro Ile Ile Tyr Pro Cys Ser Ser
 340 345 350
 Lys Glu Phe Lys Arg Ala Phe Met Arg Ile Leu Gly Cys Gln Cys Arg
 355 360 365
 Gly Gly Arg Arg Arg Arg Arg Arg Arg Arg Leu Gly Ala Cys Ala Tyr
 370 375 380
 Thr Tyr Arg Pro Trp Thr Arg Gly Gly Ser Leu Glu Arg Ser Gln Ser
 385 390 395 400
 Arg Lys Asp Ser Leu Asp Asp Ser Gly Ser Cys Met Ser Gly Gln Lys
 405 410 415
 Arg Thr Leu Pro Ser Ala Ser Pro Ser Pro Gly Tyr Leu Gly Arg Gly
 420 425 430
 Thr Gln Pro Pro Val Glu Leu Cys Ala Phe Pro Glu Trp Lys Pro Gly
 435 440 445
 Ala Leu Leu Ser Leu Pro Glu Pro Pro Gly Arg Arg Gly Arg Leu Asp
 450 455 460
 Ser Gly Pro Leu Phe Thr Phe Lys Leu Leu Gly Asp Pro Glu Ser Pro
 465 470 475 480
 Gly Thr Glu Ala Thr Ala Ser Asn Gly Gly Cys Asp Thr Thr Thr Asp
 485 490 495
 Leu Ala Asn Gly Gln Pro Gly Phe Lys Ser Asn Met Pro Leu Gly Pro
 500 505 510
 Gly His Phe
 515

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1987 base pairs
 (B) TYPE: nucleic acid

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(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GAATTCCCTC CTAGAAGCTG GAGAGAGCAG GAGCCTTCGG TGGGGCAGCT CAAAATGTAG	60
GTAAC TGCGG GCCAGGAGCA GCGCCCAGAT GCCATCGGTC CCTGCCTTTG AGCGTCGACG	120
GCTGATCTTT TGGTTTGAGG GAGAGACTGG CGCTGGAGTT TTGAATTCCG AATCATGTGC	180
AGAATCGTGA ATCTTCCCC AGCCAGGACG AATAAGACAG CGCGGAAAAG CAGATTCTCG	240
TAATTCTGGA ATTGCATGTT GCAAGGAGTC TCCTGGATCT TCGCACCCAG CTTCGGGTAC	300
GGGAGGGAGT CCGGGTCCCG GCTAGGCCAG CCCGCAGGTG GAGAGGGTCC CCGGCAGCCC	360
CGCGCGCCCC TGGCCATGTC TTTAATGCCC TGCCCCCTCA TGTGGCCTTC TGAGGGTTCC	420
CAGGGCTGGC CAGGGTTGTC TCCCACCCGC GCGCGCCGTC TCACCCCCAG CCAAACCCAC	480
CTGGCAGGGC TCCCTCCAGA AGAGACCTTT TGATTCCCCG CTCCCGCGCT CCCGCCTCCG	540
CGCCAGCCCG GGAGGTGGCC CTGGACAGCC GGACCTCGCC CGGCCCCGGC TGGGACCATG	600
GTGTTTCTCT CGGGAAATGC TTCCGACAGC TCCAAC TGCA CCCAACCGCC GGCACCGGTG	660
AACATTTCCA AGGCCATTCT GCTCGGGGTG ATCTTGGGGG GCCTCATTCT TTTCGGGGTG	720
CTGGGTAACA TCCTAGTGAT CCTCTCCGTA GCCTGTCACC GACACCTGCA CTCAGTCACG	780
CACTACTACA TCGTCAACCG CTAGTGGCGG TGGCCGACCT CCTGCTCACC TCCACGGTGC	840
TGCCCTTCTC CGCCATCTTC GAGGTCCTAG GCTACTGGGC CTTCGGCAGG GTCTTCTGCA	900
ACATCTGGGC GGCAGTGAT GTGCTGTGCT GCACCGCGTC CATCATGGGC CTCTGCATCA	960
TCTCCATCGA CCGCTACATC GCGGTGAGCT ACCCGCTGCG CTACCCAACC ATCGTCACCC	1020
AGAGGAGGGG TCTCATGGCT CTGCTCTGCG TCTGGGCACT CTCCCTGGTC ATATCCATTG	1080
GACCCCTCTT CGGCTGGAGG CAGCCGGCCC CCGAGGACGA GACCATCTGC CAGATCAACG	1140
AGGAGCCGGG CTACGTGCTC TTCTCGGCTC TGGGCTCCTT CTACCTGCCT CTGGCCATCA	1200
TCCTGGTCAT GTACTGCCGC GTCTACGTGG TGGCCAAGAG GGAGAGCCGG GGCCTCAAGT	1260
CTGGCCTCAA GACCGACAAG TCGGACTCGG AGCAAGTGAC GCTCCGCATC CATCGGAAAA	1320

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ACGCCCCGGC AGGAGGCAGC GGGATGGCCA GCGCCAAGAC CAAGACGCAC TTCTCAGTGA 1380
 GGCTCCTCAA GTTCTCCCGG GAGAAGAAAG CGGCCAAAAC GCTGGGCATC GTGGTCGGCT 1440
 GCTTCGTCTT CTGCTGGCTG CCTTTTTTCT TAGTCATGCC CATTGGGTCT TTCTTCCCTG 1500
 ATTTCAAGCC CTCTGAAACA GTTTTTTAAA TAGTATTTTG GCTCGGATAT CTAAACAGCT 1560
 GCATCAACCC CATCATATAC CCATGCTCCA GCCAAGAGTT CAAAAAGGCC TTTCAGAATG 1620
 TCTTGAGAAT CCAGTGTCTC CGCAGAAAGC AGTCGCTAGT TCCAAACATG CCCTGGGCTA 1680
 CACCCTGCAC CCGCCAGCC AGGCCGTGGA AGGGCAACAC AAGGACATGG TCGCATCCC 1740
 CGTGGGATCA AGAGAGACCT TCTACAGGAT CTCCAAGACG GATGGCGTTT GTGAATGGAA 1800
 ATTTTTCTCT TCCATGCCCC GTGGATCTGC CAGGATTACA GTGTCAAAG ACCAATCCTC 1860
 CTGTACCACA GCGCGGTGA GAAGTAAAG CTTTTTGCAG GTCTGCTGCT GTGTAGGGCC 1920
 CTCAACCCCC AGCCTTGACA AGAACCATCA AGTTCCAACC ATTAAGGTCC ACACCATCTC 1980
 CCTCAGT 1987

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AATTCCCTCC TAGAAGCTGG AGAGAGCAGG AGCCTTCGGT GGGGCAGCTC AAAATGTAGG 60
 TAACTGCGGG CCAGGAGCAG CGCCAGATG CCATCGGTCC CTGCCTTTGA GCGTCGACGG 120
 CTGATCTTTT GGTTTGAGGG AGAGACTGGC GCTGGAGTTT TGAATTCCGA ATCATGTGCA 180
 GAATCGTGAA TCTTCCCCCA GCCAGGACGA ATAAGACAGC GCGGAAAAGC AGATTCTCGT 240
 AATTCTGGAA TTGCATGTTG CAAGGAGTCT CCTGGATCTT CGCAGCCAGC TTCGGGTACG 300
 GGAGGGAGTC CGGGTCCCGG CTAGGCCAGC CCGCAGGTGG AGAGGGTCCC CGGCAGCCCC 360
 GCGCGCCCCCT GGCCATGTCT TTAATGCCCT GCCCCTTCAT GTGGCCTTCT GAGGGTTCCC 420

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AGGGCTGGCC AGGGTTGTCT CCCACCCGCG CGCGCCGTCT CACCCCCAGC CAAACCCACC	480
TGGCAGGGCT CCCTCCAGAA GAGACCTTTT GATTCCCGGC TCCCGCGCTC CCGCCTCCGC	540
GCCAGCCCGG GAGGTGGCCC TGGACAGCCG GACCTCGCCC GGCCCCGGCT GGGACCATGG	600
TGTTTCTCTC GGGAAATGCT TCCGACAGCT CCAACTGCAC CCAACCGCCG GCACCGGTGA	660
ACATTTCCAA GGCCATTCTG CTCGGGGTGA TCTTGGGGG CCTCATTCTT TTCGGGGTGC	720
TGGGTAACAT CCTAGTGATC CTCTCCGTAG CCTGTACCCG ACACCTGCAC TCAGTCACGC	780
ACTACTACAT CGTCAACCTG GCGGTGGCCG ACCTCCTGCT CACCTCCACG GTGCTGCCCT	840
TCTCCGCCAT CTTGAGGTC CTAGGCTACT GGGCCTTCGG CAGGGTCTTC TGCAACATCT	900
GGGCGGCAGT GGATGTGCTG TGCTGCACCG CGTCCATCAT GGGCCTCTGC ATCATCTCCA	960
TCGACCGCTA CATCGGCGTG AGCTACCCGC TGCCTACCC AACCATCGTC ACCCAGAGGA	1020
GGGGTCTCAT GGCTCTGCTC TGCGTCTGGG CACTCTCCCT GGTCATATCC ATTGGACCCC	1080
TCTTCGGCTG GAGGCAGCCG GCCCCGAGG ACGAGACCAT CTGCCAGATC AACGAGGAGC	1140
CGGGCTACGT GCTCTTCTCG GCTCTGGGCT CTTCTACCT GCCTCTGGCC ATCATCTGG	1200
TCATGTACTG CCGCGTCTAC GTGTGGCCA AGAGGGAGAG CCGGGGCCCTC AAGTCTGGCC	1260
TCAAGACCGA CAAGTCGGAC TCGGAGCAAG TGACGCTCCG CATCCATCGG AAAAACGCCC	1320
CGGCAGGAGG CAGCGGGATG GCCAGCGCCA AGACCAAGAC GCACCTCTCA GTGAGGCTCC	1380
TCAAGTTCTC CCGGGAGAAG AAAGCGGCCA AAACGCTGGG CATCGTGGTC GGCTGCTTCG	1440
TCCTCTGCTG GCTGCCTTTT TTCTTAGTCA TGCCCATTTG GTCTTTCTTC CCTGATTTCA	1500
AGCCCTCTGA AACAGTTTTT AAAATAGTAT TTTGGCTCGG ATATCTAAAC AGCTGCATCA	1560
ACCCCATCAT ATACCCATGC TCCAGCCAAG AGTTCAAAAA GGCCTTTCAG AATGTCTTGA	1620
GAATCCAGTG TCTCTGCAGA AAGCAGTCTT CCAAACATGC CCTGGGCTAC ACCCTGCACC	1680
CGCCAGCCA GGCCGTGGAA GGGCAACACA AGGACATGGT GCGCATCCCC GTGGGATCAA	1740
GAGAGACCTT CTACAGGATC TCCAAGACGG ATGGCGTTTG TGAATGGAAA TTTTCTCTT	1800
CCATGCCCCG TGGATCTGCC AGGATTACAG TGTCCAAAGA CCAATCCTCC TGTACCACAG	1860
CCCGGTGAG AAGTAAAGC TTTTGCAGG TCTGCTGCTG TGTAGGGCCC TCAACCCCCA	1920
GCCTTGACAA GAACCATCAA GTTCCAACCA TTAAGGTCCA CACCATCTCC CTCAGTGAGA	1980
ACGGGGAGGA AGTCTAG	1997

(2) INFORMATION FOR SEQ ID NO:28:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 466 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```

Met Val Phe Leu Ser Gly Asn Ala Ser Asp Ser Ser Asn Cys Thr Gln
1           5           10           15

Pro Pro Ala Pro Val Asn Ile Ser Lys Ala Ile Leu Leu Gly Val Ile
          20           25           30

Leu Gly Gly Leu Ile Leu Phe Gly Val Leu Gly Asn Ile Leu Val Ile
      35           40           45

Leu Ser Val Ala Cys His Arg His Leu His Ser Val Thr His Tyr Tyr
50           55           60

Ile Val Asn Leu Ala Val Ala Asp Leu Leu Leu Thr Ser Thr Val Leu
65           70           75           80

Pro Phe Ser Ala Ile Phe Glu Val Leu Gly Tyr Trp Ala Phe Gly Arg
          85           90           95

Val Phe Cys Asn Ile Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala
          100          105          110

Ser Ile Met Gly Leu Cys Ile Ile Ser Ile Asp Arg Tyr Ile Gly Val
      115          120          125

Ser Tyr Pro Leu Arg Tyr Pro Thr Ile Val Thr Gln Arg Arg Gly Leu
130          135          140

Met Ala Leu Leu Cys Val Trp Ala Leu Ser Leu Val Ile Ser Ile Gly
145          150          155          160

Pro Leu Phe Gly Trp Arg Gln Pro Ala Pro Glu Asp Glu Thr Ile Cys
          165          170          175

Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu Gly Ser
          180          185          190

Phe Tyr Leu Pro Leu Ala Ile Ile Leu Val Met Tyr Cys Arg Val Tyr
195          200          205

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Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly Leu Lys Thr
 210 215 220
 Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile His Arg Lys Asn
 225 230 235 240
 Ala Pro Ala Gly Gly Ser Gly Met Ala Ser Ala Lys Thr Lys Thr His
 245 250 255
 Phe Ser Val Arg Leu Leu Lys Phe Ser Arg Glu Lys Lys Ala Ala Lys
 260 265 270
 Thr Leu Gly Ile Val Val Gly Cys Phe Val Leu Cys Trp Leu Pro Phe
 275 280 285
 Phe Leu Val Met Pro Ile Gly Ser Phe Phe Pro Asp Phe Lys Pro Ser
 290 295 300
 Glu Thr Val Phe Lys Ile Val Phe Trp Leu Gly Tyr Leu Asn Ser Cys
 305 310 315 320
 Ile Asn Pro Ile Ile Tyr Pro Cys Ser Ser Gln Glu Phe Lys Lys Ala
 325 330 335
 Phe Gln Asn Val Leu Arg Ile Gln Cys Leu Cys Arg Lys Gln Ser Ser
 340 345 350
 Lys His Ala Leu Gly Tyr Thr Leu His Pro Pro Ser Gln Ala Val Glu
 355 360 365
 Gly Gln His Lys Asp Met Val Arg Ile Pro Val Gly Ser Arg Glu Thr
 370 375 380
 Phe Tyr Arg Ile Ser Lys Thr Asp Gly Val Cys Glu Trp Lys Phe Phe
 385 390 395 400
 Ser Ser Met Pro Arg Gly Ser Ala Arg Ile Thr Val Ser Lys Asp Gln
 405 410 415
 Ser Ser Cys Thr Thr Ala Arg Val Arg Ser Lys Ser Phe Leu Gln Val
 420 425 430
 Cys Cys Cys Val Gly Pro Ser Thr Pro Ser Leu Asp Lys Asn His Gln
 435 440 445
 Val Pro Thr Ile Lys Val His Thr Ile Ser Leu Ser Glu Asn Gly Glu
 450 455 460
 Glu Val
 465

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1776 base pairs
 (B) TYPE: nucleic acid

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(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTCCCTGCCG GCCGCTCGTT CTGTGCCCCG GCCCGGCCAC CGACGGCCGG CGTTGAGATG	60
ACTTTCGCG ATCTCCTGAG CGTCAGTTTC GAGGGACCCC GCCCGGACAG CAGCGCAGGG	120
GGCTCCAGCG CGGGCGGCGG CGGGGGCGGC GCGGGCGGCG CGGCCCCCTC GGAGGGCCCC	180
GCGGTGGGCG GCGTGCCGGG GGGCGCGGGC GCGGCGGCG GCGTGGTGGG CGCAGGCAGC	240
GGCGAGGACA ACCGGAGCTC CGCGGGGGAG CCGGGGAGCG CGGCGCGGG CGGCGACGTG	300
AATGGCACGG CGGCCGTCGG GGGACTGGTG GTGAGCGCGC AGGGCGTGGG CGTGGGCGTC	360
TTCTTGCCAG CCTTCATCCT TATGGCCGTG GCAGGTAACC TGCTTGTCAT CCTCTCAGTG	420
GCCTGCAACC GCCACCTGCA GACCGTCACC AACTATTTCA TCGTGAACCT GGCCGTGGCC	480
GACCTGCTGC TGAGCGCCAC CGTACTGCCC TTCTCGGCCA CCATGGAGGT TCTGGGCTTC	540
TGGGCCTTTG GCCGCGCCTT CTGCGACGTA TGGGCCGCCG TGGACGTGCT GTGCTGCACG	600
GCCTCCATCC TCAGCCTCTG CACCATCTCC GTGGACCGGT ACGTGGGCGT GCGCCACTCA	660
CTCAAGTACC CAGCCATCAT GACCGAGCGC AAGGCGGCCG CCATCCTGGC CCTGCTCTGG	720
GTGCTAGCCC TGGTGGTGTC CGTAGGGCCC CTGCTGGGCT GGAAGGAGCC CGTGCCCCCT	780
GACGAGCGCT TCTGCGGTAT CACCGAGGAG GCGGGCTACG CTGTCTTCTC CTCCGTGTGC	840
TCCTTCTACC TGCCCATGGC GGTCAATCGTG GTCATGTACT GCCGCGTGTA CGTGGTCGCG	900
CGCAGCACCA CGCGCAGCCT CGAGGCGGGC GTCAAGCGCG AGCGAGGCAA GGCCTCCGAG	960
GTGGTGCTGC GCATCCACTG TCGCGGCGCG GCCACGGGCG CCGACGGGGC GCACGGCATG	1020
CGCAGCGCCA AGGGCCACAC CTTCCGCAGC TCGCTCTCCG TCGCCTGCT CAAGTTCTCC	1080
CGTGAGAAGA AAGCGGCCAA GACTCTGGCC ATCGTCGTGG GTGTCTTCGT GCTCTGCTGG	1140
TTCCCTTTCT TCTTTGTCCT GCCGCTCGGC TCCTTGTTCC CGCAGCTGAA GCCATCGGAG	1200
GGCGTCTTCA AGGTCATCTT CTGGCTCGGC TACTTCAACA GCTGCGTGAA CCCGCTCATC	1260
TACCCCTGTT CCAGCCGCGA GTTCAAGCGC GCCTTCCTCC GTCTCCTGCG CTGCCAGTGC	1320

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CGTCGTCGCC GCGCGCCGCG CCCTCTCTGG CGTGTCTACG GCCACCACTG GCGGGCCTCC 1380
 ACCAGCGGCC TGC GCCAGGA CTGCGCCCCG AGTTGCGGCG ACGCGCCCCC CGGAGCGCCG 1440
 CTGGCCCTCA CCGCGCTCCC CGACCCCGAC CCCGAACCCC CAGGCACGCC CGAGATGCAG 1500
 GCTCCGGTCG CCAGCCGTCG AAAGCCACCC AGCGCCTTCC GCGAGTGGAG GCTGCTGGGG 1560
 CCGTTCCGGA GACCCACGAC CCAGCTGCGC GCCAAAGTCT CCAGCCTGTC GCACAAGATC 1620
 CGCGCCGGGG GCGCGCAGCG CGCAGAGGCA GCGTGCGCC AGCGCTCAGA GGTGGAGGCT 1680
 GTGTCCCTAG GCGTCCCACA CGAGGTGGCC GAGGGCGCCA CCTGCCAGGC CTACGAATTG 1740
 GCCGACTACA GCAACCTACG GGAGACCGAT ATTTAA 1776

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met Thr Phe Arg Asp Leu Leu Ser Val Ser Phe Glu Gly Pro Arg Pro
 1 5 10 15
 Asp Ser Ser Ala Gly Gly Ser Ser Ala Gly Gly Gly Gly Gly Gly Ala
 20 25 30
 Gly Gly Ala Ala Pro Ser Glu Gly Pro Ala Val Gly Gly Val Pro Gly
 35 40 45
 Gly Ala Gly Gly Gly Gly Gly Val Val Gly Ala Gly Ser Gly Glu Asp
 50 55 60
 Asn Arg Ser Ser Ala Gly Glu Pro Gly Ser Ala Gly Ala Gly Gly Asp
 65 70 75 80
 Val Asn Gly Thr Ala Ala Val Gly Gly Leu Val Val Ser Ala Gln Gly
 85 90 95
 Val Gly Val Gly Val Phe Leu Ala Ala Phe Ile Leu Met Ala Val Ala

100

105

110

Gly	Asn	Leu	Leu	Val	Ile	Leu	Ser	Val	Ala	Cys	Asn	Arg	His	Leu	Gln
		115						120				125			
Thr	Val	Thr	Asn	Tyr	Phe	Ile	Val	Asn	Leu	Ala	Val	Ala	Asp	Leu	Leu
	130					135					140				
Leu	Ser	Ala	Thr	Val	Leu	Pro	Phe	Ser	Ala	Thr	Met	Glu	Val	Leu	Gly
145					150					155					160
Phe	Trp	Ala	Phe	Gly	Arg	Ala	Phe	Cys	Asp	Val	Trp	Ala	Ala	Val	Asp
				165					170					175	
Val	Leu	Cys	Cys	Thr	Ala	Ser	Ile	Leu	Ser	Leu	Cys	Thr	Ile	Ser	Val
			180					185					190		
Asp	Arg	Tyr	Val	Gly	Val	Arg	His	Ser	Leu	Lys	Tyr	Pro	Ala	Ile	Met
		195					200					205			
Thr	Glu	Arg	Lys	Ala	Ala	Ala	Ile	Leu	Ala	Leu	Leu	Trp	Val	Val	Ala
	210					215					220				
Leu	Val	Val	Ser	Val	Gly	Pro	Leu	Leu	Gly	Trp	Lys	Glu	Pro	Val	Pro
225					230					235					240
Pro	Asp	Glu	Arg	Phe	Cys	Gly	Ile	Thr	Glu	Glu	Ala	Gly	Tyr	Ala	Val
				245					250					255	
Phe	Ser	Ser	Val	Cys	Ser	Phe	Tyr	Leu	Pro	Met	Ala	Val	Ile	Val	Val
			260					265					270		
Met	Tyr	Cys	Arg	Val	Tyr	Val	Val	Ala	Arg	Ser	Thr	Thr	Arg	Ser	Leu
		275					280					285			
Glu	Ala	Gly	Val	Lys	Arg	Glu	Arg	Gly	Lys	Ala	Ser	Glu	Val	Val	Leu
	290					295					300				
Arg	Ile	His	Cys	Arg	Gly	Ala	Ala	Thr	Gly	Ala	Asp	Gly	Ala	His	Gly
305					310					315					320
Met	Arg	Ser	Ala	Lys	Gly	His	Thr	Phe	Arg	Ser	Ser	Leu	Ser	Val	Arg
				325					330					335	
Leu	Leu	Lys	Phe	Ser	Arg	Glu	Lys	Lys	Ala	Ala	Lys	Thr	Leu	Ala	Ile
			340					345					350		
Val	Val	Gly	Val	Phe	Val	Leu	Cys	Trp	Phe	Pro	Phe	Phe	Phe	Val	Leu
		355					360					365			
Pro	Leu	Gly	Ser	Leu	Phe	Pro	Gln	Leu	Lys	Pro	Ser	Glu	Gly	Val	Phe
	370					375					380				
Lys	Val	Ile	Phe	Trp	Leu	Gly	Tyr	Phe	Asn	Ser	Cys	Val	Asn	Pro	Leu
385					390					395					400

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Ile	Tyr	Pro	Cys	Ser	Ser	Arg	Glu	Phe	Lys	Arg	Ala	Phe	Leu	Arg	Leu
				405					410						415
Leu	Arg	Cys	Gln	Cys	Arg	Arg	Arg	Arg	Arg	Arg	Arg	Pro	Leu	Trp	Arg
			420					425					430		
Val	Tyr	Gly	His	His	Trp	Arg	Ala	Ser	Thr	Ser	Gly	Leu	Arg	Gln	Asp
		435					440					445			
Cys	Ala	Pro	Ser	Ser	Gly	Asp	Ala	Pro	Pro	Gly	Ala	Pro	Leu	Ala	Leu
		450				455					460				
Thr	Ala	Leu	Pro	Asp	Pro	Asp	Pro	Glu	Pro	Pro	Gly	Thr	Pro	Glu	Met
465					470					475					480
Gln	Ala	Pro	Val	Ala	Ser	Arg	Arg	Lys	Pro	Pro	Ser	Ala	Phe	Arg	Glu
				485					490					495	
Trp	Arg	Leu	Leu	Gly	Pro	Phe	Arg	Arg	Pro	Thr	Thr	Gln	Leu	Arg	Ala
			500					505					510		
Lys	Val	Ser	Ser	Leu	Ser	His	Lys	Ile	Arg	Ala	Gly	Gly	Ala	Gln	Arg
		515					520					525			
Ala	Glu	Ala	Ala	Cys	Ala	Gln	Arg	Ser	Glu	Val	Glu	Ala	Val	Ser	Leu
		530				535					540				
Gly	Val	Pro	His	Glu	Val	Ala	Glu	Gly	Ala	Thr	Cys	Gln	Ala	Tyr	Glu
545					550					555					560
Leu	Ala	Asp	Tyr	Ser	Asn	Leu	Arg	Glu	Thr	Asp	Ile				
				565					570						

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GAATCCCGAC CTGGAC

16

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGATCCTCAG GGTC

14

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCATGGTGTT TCTCTCGGG

19

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GACGCGGCAG TACATGAC

18

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(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

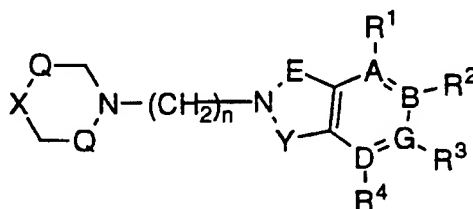
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTCATGATGG CTGGGTACTT G

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WHAT IS CLAIMED IS:

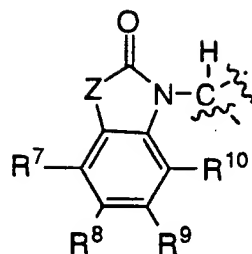
1. A compound having the formula:



and a pharmaceutically acceptable salt, prodrug, polymorph, or metabolite thereof wherein:

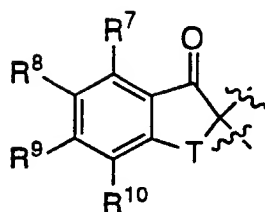
- n is an integer from 3 to 5;
 Y represents carbonyl, sulphonyl, -CO-CH₂-, or -CO-NR¹²-;
 R¹² is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted phenyl;
 E is carbonyl or sulphonyl;
 A, B, G, D are independently carbon or nitrogen;
 R¹-R⁴ are independently selected from the group consisting of hydrogen; halogen; nitro; amino; substituted or unsubstituted lower alkyl; perhalogenated lower alkyl; substituted or unsubstituted lower alkoxy; sulfonyl alkyl; and substituted or unsubstituted aryl or heteroaryl, with the proviso that if any of A, B, G, or D is a nitrogen, then the substituent R group is not present;
 Q is, independently, (-CH₂)_r, -NH-, S, or O;
 r is 0-3; and
 X is
 a)

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, or

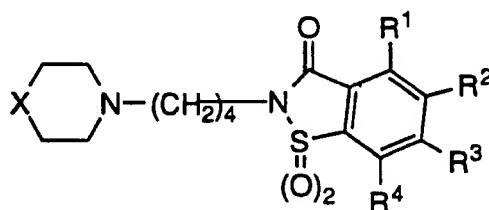
b)



T is nitrogen, carbon, lower alkylene of one to three carbons or lower alkenylene of one to three carbons;

R⁷-R¹⁰ are independently selected from the group consisting of hydrogen, lower alkyl, lower alkenyl, and lower alkoxy; and Z is O, S, CH₂, CH₂O, OCH₂, SCH₂, lower alkylene, lower alkenylene, NH, or NMe.

2. The compound of Claim 1 having the structure:

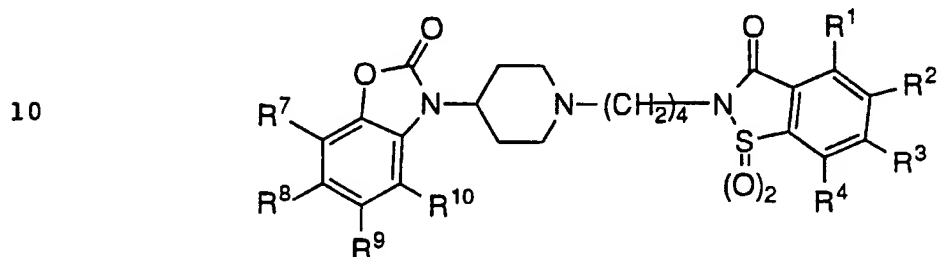


and a pharmaceutically acceptable salt, prodrug, polymorph, or metabolite thereof wherein all substituents are as defined in Claim 1.

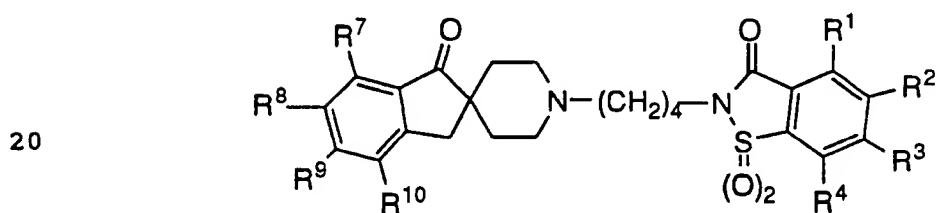
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3. The compound of Claim 2 which is a benzoxazoliny
 piperidine substituted butyl saccharine or a spiro 1-oxo-2H-indenyl
 piperidine substituted butyl saccharine.

4. The compound of Claim 3 selected from:



15 and



wherein all substituents are as defined in Claim 1.

25 5. A compound selected from:

1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-
 1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(3,4-dihydro-2-oxo-(1H)-quinolin-1-yl)-
 piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

30 1,1-Dioxido-2-(4-(4-(2-oxo-3,1,4-benzoxazinyl)-piperidin-1-yl)-butyl)-
 1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-
 1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-
 butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;

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- 6-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5 1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-7-nitro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-(1H)-quinolin-1-yl)-piperidin-1-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-
10 5-nitro-1,2-benzisothiazol-3(2H)-one;
6-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
15 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-7-nitro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5-chloro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-
20 butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(3a-(R)-8a-(S)-2-oxo-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxonaphth[2,3-d]oxazoliny)-piperidin-1-yl)-
25 butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-5-phenyl-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
30 1,1-Dioxido-2-(4-(4-(6-methoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(6-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(4-(5-ethylsulfonyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-oxazolo[4,5-b]pyridyl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5 1,1-Dioxido-2-(4-(4-(7-carbethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5-*tert*-butyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5,7-dimethyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
10 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4'-(3,4-dihydro-1-oxonaphthalene)-2(1H)-spiropiperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one;
15 4-(3,4-Dihydro-6-methyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide;
4-(Spiro(piperidine-4,6'-[6H]thieno[2,3-b]thiopyran-4'(5'H)-one-1'-yl)-butylphthalimide;
4-(Spiro[benzothiazol-2(3H),4'-piperidin-1'-yl)-butylphthalimide;
20 4-(3,4-Dihydro-6-methoxy-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide;
4-(3,4-Dihydro-6-methanesulfonylamidyl-spiro[2H-1-benzopyran-2,4'-piperidine-4(3H)-one]-1'-yl)-butylphthalimide;
1,1-Dioxido-2-(4-(spiro[benzothiazol-2(3H),4'-piperidin-1'-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
25 4-(6-Trifluoromethyl-spiro[benzothiazol-2(3H),4'-piperidin-1'-yl)-butylphthalimide;
1,1-Dioxido-2-(4-(spiro[benzofuran-2(3H),4'-piperidin]-1'-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
30 4-(Spiro[benzofuran-2(3H),4'-piperidin]-1'-yl)-butylphthalimide;
4-(Spiro[2H-1,3-benzoxazine-2,4'-piperidin]-1'-yl)-butylphthalimide;
3,3-Dioxido-1,2-dehydro-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-naphth[1,2-d]isothiazol-1-one;

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- 1,1-Dioxido-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-4-methoxy-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-7-methoxy-1,2-benzisothiazol-3(2H)-one;
5 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-methoxy-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-6-methoxy-1,2-benzisothiazol-3(2H)-one;
10 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-methyl-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one;
15 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-6-nitro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-isothiazolo[5,4-c]pyridin-3(2H)-one;
20 1,1-Dioxido-2-(4-(spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-isothiazolo[5,4-b]pyridin-3(2H)-one ;
1,1-Dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazolinyl)-piperidin-1-yl)-butyl)-5-nitro-1,2-benzisothiazol-3(2H)-one;
25 2-(4-(Spiro(1,3-dihydro-1-oxo-2H-indene-2,4'-piperidin-1'-yl)butyl)-pyrrolo[3,4b]pyridin-5,7(1H)-dione;
1,1-Dioxido-2,3-dihydro-2-(4-(spiro[2H-indene-2,4'-piperidine-1(3H)-one]-1'-yl)-butyl)-naphth[1,8-de]isothiazin-3-one; and
1,1-Dioxido-2-(4-(spiro[3-oxo-phthalan-1,4'-piperidin-1'-yl)butyl)-1,2-benzisothiazol-3(2H)-one;
30 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-3-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-4-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 5 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methoxy-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-chloro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 10 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-fluoro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-6-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;
- 15 1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;
- 20 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-1H-3,4-dihydroquinazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
- 25 2-(4-Hydroxybutyl)-1,1-dioxido-5,6-dihydro-[1,4]dioxino[2,3-d]benzisothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-ethoxy-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-ethoxy-1,2-benzothiazol-3(2H)-one;
- 30 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-ethyl-1,2-benzothiazol-3(2H)-one;
- 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-ethyl-1,2-benzothiazol-3(2H)-one;

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- 1,1-Dioxido-2-(4-(4-(spiro-1,3-dihydro-1-oxo-2H-indenyl)-2,4'-piperidin-1'-yl)-butyl)-5-(2-propyl)-1,2-benzothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-(2-propyl)-1,2-benzothiazol-3(2H)-one;
5 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-6-nitro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-fluoro-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-methyl-1,2-benzisothiazol-3(2H)-one;
10 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-bromo-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-trifluoromethyl-1,2-benzisothiazol-3(2H)-one;
15 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-trifluoromethoxy-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-1-naphth[1,2-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5,6,7,8-tetrahydro-2-oxo-3-naphth[2,3-d]oxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
20 1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(5-carbomethoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
25 1,1-Dioxido-2-(4-(4-(6-fluoro-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(4-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(4-methoxy-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
30 5-Chloro-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Methylthio-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

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5-Ethoxy-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Chloro-1,1-dioxido-2-(4-(4-(6-fluoro-2-oxo-3-benzoxazoliny)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5 4-Methyl-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-
butyl)-1,2-benzisothiazol-3(2H)-one;
4-Chloro-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-
butyl)-1,2-benzisothiazol-3(2H)-one;
4-Ethoxy-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-
10 yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
4-(2-Propyloxy)-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Methoxy-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-benzoxazoliny)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
15 1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-6-
methylsulfonyl-1,2-benzisothiazol-3(2H)-one;
5-Methoxy-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-
yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5-
20 methylsulfonyl-1,2-benzisothiazol-3(2H)-one;
5-Methylthio-1,1-dioxido-2-(4-(4-(2-oxo-3-benzoxazoliny)-piperidin-
1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
5-Methylsulfonyl-1,1-dioxido-2-(4-(4-(6-methyl-2-oxo-3-
benzoxazoliny)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one; or
25 1,1-Dioxido-2-(4-(4-(2-oxo-1-oxazolo[5,4-b]pyridyl)-piperidin-1-yl)-
butyl)-1,2-benzisothiazol-3(2H)-one;
and a pharmaceutically acceptable salt, prodrug, polymorph, or
metabolite thereof.

30

6. The compound of Claim 5 selected from:

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-2H-benzimidazolin-1-yl)-
piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;
1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-3-methyl-2H-benzimidazolin-
1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

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1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-chloro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-1,2-benzisothiazol-3(2H)-one;

5 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-fluoro-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;

1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-5-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one;

10 1,1-Dioxido-2-(4-(4-(1,3-dihydro-2-oxo-6-methyl-2H-benzimidazolin-1-yl)-piperidin-1-yl)-butyl)-5-chloro-1,2-benzisothiazol-3(2H)-one; or

1,1-Dioxido-2-(4-(4-(5-methyl-2-oxo-3-benzoxazoliny)-piperidin-1-yl)-butyl)-5,6-dihydro-[1,4]dioxino[2,3-e]benzisothiazol-3(2H)-one; and a pharmaceutically acceptable salt, prodrug, polymorph, or metabolite thereof.

15

7. A pharmaceutical composition comprising a therapeutically effective amount of the compound of Claim 1 and a pharmaceutically acceptable carrier.

20

8. The composition of Claim 7 which further comprises a therapeutically effective amount of a testosterone 5-alpha reductase inhibitor.

25

9. The composition of Claim 8, wherein the testosterone 5-alpha reductase inhibitor is a type 1, a type 2, a type 1 and a type 2 or a dual type 1 and type 2 testosterone 5-alpha reductase inhibitor.

30

10. The composition of Claim 9, wherein the testosterone 5-alpha reductase inhibitor is a type 2 testosterone 5-alpha reductase inhibitor.

11. The composition of Claim 10, wherein the testosterone 5-alpha reductase inhibitor is finasteride.

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12. A method of treating benign prostatic hyperplasia in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of the compound of Claim 1.

5 13. The method of Claim 12 wherein the compound is administered in combination with a therapeutically effective amount of a testosterone 5-alpha reductase inhibitor.

10 14. The method of Claim 13, wherein the testosterone 5-alpha reductase inhibitor is finasteride.

15 15. The method of Claim 12, wherein the compound additionally does not cause a fall in blood pressure at dosages effective for treating benign prostatic hyperplasia.

 16. A method of treating benign prostatic hyperplasia which comprises administering a therapeutically effective amount of the composition of claim 7.

20 17. A method of inhibiting contraction of prostate tissue in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of the compound of Claim 1.

25 18. The method of Claim 17 wherein the compound is administered in combination with a therapeutically effective amount of a testosterone 5-alpha reductase inhibitor.

30 19. The method of Claim 18, wherein the testosterone 5-alpha reductase inhibitor is finasteride.

 20. The method of Claim 17, wherein the compound additionally does not cause a fall in blood pressure at dosages effective for treating benign prostatic hyperplasia.

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- 5 21. A method of using the compound of Claim 1 to selectively antagonize the human $\alpha 1C$ adrenergic receptor which comprises exposing the human $\alpha 1C$ adrenergic receptor to an antagonistically effective amount of said compound.
- 10 22. A method of preparing an antagonist of the human $\alpha 1C$ adrenergic receptor which comprises alkylating a halogenated butyl saccharine with a deprotected spiropiperidine or a deprotected benzoxazolinyl piperidine
23. The use of the compound of Claim 1 in the preparation of a medicament for the treatment of benign prostatic hyperplasia.
- 15 24. The use of the compound of Claim 1 in the preparation of a medicament for inhibiting contraction of prostate tissue.
- 20 25. A drug which is useful for treating benign prostatic hyperplasia, the effective ingredient of the said drug being a compound as claimed in Claim 1.
- 25 26. A drug which is useful for inhibiting contraction of prostate tissue, the effective ingredient of the said drug being a compound as claimed in Claim 1.

30

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1 GCCGCGTCTA CGTGGTGGCC AAGAGGGAGA GCCGGGGCCT CAAGTCTGGC CTCAAGACCG 60
 61 ACAAGTCGGA CTCGGAGCAA GTGACGCTCC GCATCCATCG GAAAAACGCC CCGGCAGGAG 120
 121 GCAGCGGGAT GGCCAGCGCC AAGACCAAGA CGCACTTCTC AGTGAGGCTC CTCAAGTTCT 180
 181 CCCGGGAGAA GAAAGCGGCC AAAACGCTGG GCATCGTGGT CGGCTGCTTC GTCCT 235

FIG.1

h α 1c:Arg Val Tyr Val Val Ala Lys Arg Glu Ser
 | | | | | | | | | |
 b α 1c: ...Leu Val Met Tyr Cys Arg Val Tyr Val Val Ala Lys Arg Glu Ser

h α 1C: ...Arg Gly Leu Lys Ser Gly Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln
 | | | | | | | | | |
 b α 1C: ...Arg Gly Leu Lys Ser Gly Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln

h α 1C: ...Val Thr Leu Arg Ile His Arg Lys Asn Ala Pro Ala Gly Gly Ser Gly
 | | | | | | | | | |
 b α 1C: ...Val Thr Leu Arg Ile His Arg Lys Asn Ala Gln Val Gly Gly Ser Gly

h α 1c: ...Met Ala Ser Ala Lys Thr Lys Thr His Phe Ser Val Arg Leu Leu Lys
 . . | | | . | | | | | |
 b α 1C: ...Val Thr Ser Ala Lys Asn Lys Thr His Phe Ser Val Arg Leu Leu Lys

h α 1C: ...Phe Ser Arg Glu Lys Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly
 | | | | | | | | | |
 b α 1C: ...Phe Ser Arg Glu Lys Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly

h α 1C: ...Cys Phe Val Leu.....
 | | | |
 b α 1C: ...Cys Phe Val Leu Cys Trp Leu Pro Phe Leu Val Met Pro

FIG.2

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1 GAATTCCTC CTAGAAGCTG GAGAGAGCAG GAGCCTTCGG TGGGGCAGCT CAAAATGTAG 60
61 GTAACGCGG GCCAGGAGCA GCGCCAGAT GCCATCGGTC CCTGCCTTTG AGCGTCGACG 120
121 GCTGATCTTT TGGTTTGAGG GAGAGACTGG CGCTGGAGTT TTGAATTCCG AATCATGTGC 180
181 AGAATCGTGA ATCTTCCCC AGCCAGGACG AATAAGACAG CGCGGAAAAG CAGATTCTCG 240
241 TAATTCTGGA ATTGCATGTT GCAAGGAGTC TCCTGGATCT TCGCACCCAG CTTCGGGTAC 300
301 GGGAGGGAGT CCGGGTCCCG GCTAGGCCAG CCCGCAGGTG GAGAGGGTCC CCGGCAGCCC 360
361 CGCGCGCCCC TGGCCATGTC TTTAATGCCC TGCCCTTCA TGTGGCCTTC TGAGGGTTCC 420
421 CAGGGCTGGC CAGGGTTGTC TCCCACCCGC GCGCGCCGTC TCACCCCCAG CCAAACCCAC 480
481 CTGGCAGGGC TCCCTCCAGA AGAGACCTTT TGATTCCCGG CTCCCGCGCT CCCGCCTCCG 540
541 CGCCAGCCCG GGAGGTGGCC CTGGACAGCC GGACCTCGCC CGGCCCCGGC TGNGGACCAT 600
601 GGTGTTTCTC TCGGGAATG CTCCGACAG CTCCAACGTC ACCCAACCGC CGGCACCGGT 660
661 GAACATTTC AAGGCCATTC TGCTCGGGT GATCTGGGG GGCCTCATTC TTTTCGGGGT 720
721 GCTGGTAAC ATCCTAGTGA TCCTCTCGT AGCCTGTAC CGACACCTGC ACTCAGTCAC 780
781 GCACTACTAC ATCGTCAACC TGGCGGTGGC CGACCTCTG CTCACCTCCA CGGTGCTGCC 840
841 CTTCTCCGCC ATCTTCGAGG TCCTAGGCTA CTGGGCCTTC GGCAGGGTCT TCTGCAACAT 900
901 CTGGGCGGCA GTGGATGTGC TGTGCTGCAC CGCGTCCATC ATGGGCCTCT GCATCATCTC 960
961 CATCGACCGC TACATCGCGG TGAGCTACCC GCTGCGCTAC CCAACCATCG TCACCCAGAG 1020
1021 GAGGGGTCTC ATGGCTCTGC TCTGCGTCTG GGCACCTCTC CTGGTCATAT CCATTGGACC 1080
1081 CCTCTTCGGC TGGAGGCAGC CGGCCCCCGA GGACGAGACC ATCTGCCAGA TCAACGAGGA 1140
1141 GCCGGGCTAC GTGCTTTCT CGGCTCTGGG CTCCTTCTAC CTGCCTCTGG CCATCATCCT 1200

FIG.3A

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1201 GGTCA GTAC TGGCGGTCT ACGTGGTGGC CAAGAGGGAG AGCCGGGGCC TCAAGTCTGG 1260
1261 CCTCAAGACC GACAAGTCGG ACTCGGAGCA AGTGACGCTC CGCATCCATC GGAAAAACGC 1320
1321 CCCGGCAGGA GGCAGCGGGA TGGCCAGCGC CAAGACCAAG ACGCACTTCT CAGTGAGGCT 1380
1381 CCTCAAGTTC TCCCGGGAGA AGAAAGCGGC CAAAACGCTG GGCATCGTGG TCGGCTGCTT 1440
1441 CGTCCTCTGC TGGCTGCCTT TTTTCTTAGT CATGCCCATT GGTCTTTCT TCCCTGATTT 1500
1501 CAAGCCCTCT GAAACAGTTT TTAAAATAGT ATTTTGGCTC GGATATCTAA ACAGCTGCAT 1560
1561 CAACCCCATC ATATACCCAT GCTCCAGCCA AGAGGGAATTCC

FIG.3B

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1 CTGATTTCAA GCCCTCTGAA ACAGTTTTTA AAATAGTATT TTGGCTCGGA TATCTAAACA 60
61 GCTGCATCAA CCCCATCATA TACCCATGCT CCAGCCAAGA GTTCAAAAAG GCCTTTCAGA 120
121 ATGTCTTGAG AATCCAGTGT CTCCGCAGAA AGCAGTCTTC CAAACATGCC CTGGGCTACA 180
181 CCCTGCACCC GCCCAGCCAG GCCGTGGAAG GGCAACACAA GGACATGGTG CGCATCCCCG 240
241 TGGGATCAAG AGAGACCTTC TACAGGATCT CCAAGACGGA TGGCGTTTGT GAATGGAAAT 300
301 TTTTCTCTTC CATGCCCCGT GGATCTGCCA GGATTACAGT GTCCAAAGAC CAATCCTCCT 360
361 GTACCACAGC CCGGGTGAGA AGTAAAAGCT TTTTGCAGGT CTGCTGCTGT GTAGGGCCCT 420
421 CAACCCCCAG CCTTGACAAG AACCATCAAG TTCCAACCAT TAAGGTCCAC ACCATCTCCC 480
481 TCAGTGAgAA CGGcGAoGAg GTtTAAGAAT TC 512

FIG.4

1 GAATTCCTC CTAGAAGCTG GAGAGAGCAG GAGCCTTCGG TGGGGCAGCT
51 CAAAATGTAG GTAAGTGGG GCCAGGAGCA GCGCCAGAT GCCATCGGTC
101 CCTGCCTTTG AGCGTCGACG GCTGATCTTT TGGTTTGAGG GAGAGACTGG
151 CGCTGGAGTT TTGAATTCG AATCATGTGC AGAATCGTGA ATCTTCCCCC
201 AGCCAGGACG AATAAGACAG CGCGGAAAAG CAGATTCTCG TAATTCTGGA
251 ATTGCATGTT GCAAGGAGTC TCCTGGATCT TCGCACCCAG CTTCGGGTAC
301 GGGAGGGAGT CCGGGTCCCG GCTAGGCCAG CCCGCAGGTG GAGAGGGTCC
351 CCGGCAGCCC CGCGCGCCCC TGGCCATGTC TTTAATGCCC TGCCCCTTCA
401 TGTGGCCTTC TGAGGGTTCC CAGGGCTGGC CAGGGTTGTC TCCCACCCGC
451 GCGCGCCGTC TCACCCCCAG CCAAACCCAC CTGGCAGGGC TCCCTCCAGA
501 AGAGACCTTT TGATTCCCGG CTCCCGCGCT CCCGCCCTCG CGCCAGCCCG
551 GGAGGTGGCC CTGGACAGCC GGACCTCGCC CGGCCCCGGC TGNGGACCAT
601 GGTGTTTCTC TCGGGAAATG CTTCCGACAG CTCCAAGTGC ACCCAACCGC
651 CGGCACCGGT GAACATTTCC AAGGCCATTG TGCTCGGGT GATCTTGGGG
701 GGCCTCATTG TTTTCGGGGT GCTGGGTAAC ATCCTAGTGA TCCTCTCCGT
751 AGCCTGTCAC CGACACCTGC ACTCAGTCAC GCACTACTAC ATCGTCAACC
801 TGGCGGTGGC CGACCTCCTG CTCACCTCCA CGGTGCTGCC CTTCTCCGCC
851 ATCTTCGAGG TCCTAGGCTA CTGGGCCTTC GGCAGGGTCT TCTGCAACAT
901 CTGGGCGGCA GTGGATGTGC TGTGCTGCAC CGCGTCCATC ATGGGCCTCT
951 GCATCATCTC CATCGACCGC TACATCGGCG TGAGCTACCC GCTGCGCTAC

FIG.5A

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1001 CCAACCATCG TCACCCAGAG GAGGGTCTC ATGGCTCTGC TCTGCGTCTG
1051 GGCACCTCTCC CTGGTCATAT CCATTGGACC CCTCTTCGGC TGGAGGCAGC
1101 CGGCCCCCGA GGACGAGACC ATCTGCCAGA TCAACGAGGA GCCGGGCTAC
1151 GTGCTCTTCT CGGCTCTGGG CTCCTTCTAC CTGCCTCTGG CCATCATCCT
1201 GGTCACTGTAC TGCCGCGTCT ACGTGGTGGC CAAGAGGGAG AGCCGGGGCC
1251 TCAAGTCTGG CCTCAAGACC GACAAGTCGG ACTCGGAGCA AGTGACGCTC
1301 CGCATCCATC GGAAAAACGC CCCGGCAGGA GGCAGCGGA TGGCCAGCGC
1351 CAAGACCAAG ACGCACTTCT CAGTGAGGCT CCTCAAGTTC TCCCGGGAGA
1401 AGAAAGCGGC CAAAACGCTG GGCATCGTGG TCGGCTGCTT CGTCCTCTGC
1451 TGGCTGCCIT TTTTCTTAGT CATGCCCATT GGGTCTTTCT TCCCTGATTT
1501 CAAGCCCTCT GAAACAGTTT TAAAATAGT ATTTGGCTC GGATATCTAA
1551 ACAGCTGCAT CAACCCCATC ATATACCAT GCTCCAGCCA AGAGTTCAAA
1601 AAGGCCTTTC AGAATGTCTT GAGAATCCAG TGTCTCCGA GAAAGCAGTC
1651 TTCAAACAT GCCCTGGGCT ACACCTGCA CCCGCCAGC CAGGCCGTGG
1701 AAGGGCAACA CAAGGACATG GTGCGCATCC CCGTGGGATC AAGAGAGACC
1751 TTCTACAGGA TCTCAAGAC GGATGGCGTT TGTGAATGGA AATTTTCTC
1801 TTCCATGCCC CGTGGATCTG CCAGGATTAC AGTGTCAAA GACCAATCCT
1851 CCTGTACCAC AGCCCGGTG AGAAGTAAA GCTTTTGA GGTCTGCTGC
1901 TGTGTAGGGC CCTCAACCCC CAGCCTTGAC AAGAACCATC AAGTTCCAAC
1951 CATTAGGTC CACACCATCT CCCTCAGTGA GAACGGCGAA GAGGTTTAAG
2001 AATTC

FIG.5B

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1 MetValPheLeuSerGlyAsnAlaSerAsp SerSerAsnCysThrGlnProProAlaPro 20
21 ValAsnIleSerLysAlaIleLeuLeuGly ValIleLeuGlyGlyLeuIleLeuPheGly 40
41 ValLeuGlyAsnIleLeuValIleLeuSer ValAlaCysHisArgHisLeuHisSerVal 60
61 ThrHisTyrTyrIleValAsnLeuAlaVal AlaAspLeuLeuLeuThrSerThrValLeu 80
81 ProPheSerAlaIlePheGluValLeuGly TyrTrpAlaPheGlyArgValPheCysAsn 100
101 IleTrpAlaAlaValAspValLeuCysCys ThrAlaSerIleMetGlyLeuCysIleIle 120
121 SerIleAspArgTyrIleGlyValSerTyr ProLeuArgTyrProThrIleValThrGln 140
141 ArgArgGlyLeuMetAlaLeuLeuCysVal TrpAlaLeuSerLeuValIleSerIleGly 160
161 ProLeuPheGlyTrpArgGlnProAlaPro GluAspGluThrIleCysGlnIleAsnGlu 180
181 GluProGlyTyrValLeuPheSerAlaLeu GlySerPheTyrLeuProLeuAlaIleIle 200
201 LeuValMetTyrCysArgValTyrValVal AlaLysArgGluSerArgGlyLeuLysSer 220
221 GlyLeuLysThrAspLysSerAspSerGlu GlnValThrLeuArgIleHisArgLysAsn 240
241 AlaProAlaGlyGlySerGlyMetAlaSer AlaLysThrLysThrHisPheSerValArg 260
261 LeuLeuLysPheSerArgGluLysLysAla AlaLysThrLeuGlyIleValValGlyCys 280
281 PheValLeuCysTrpLeuProPhePheLeu ValMetProIleGlySerPhePheProAsp 300
301 PheLysProSerGluThrValPheLysIle ValPheTrpLeuGlyTyrLeuAsnSerCys 320
321 IleAsnProIleIleTyrProCysSerSer GlnGluPheLysLysAlaPheGlnAsnVal 340
341 LeuArgIleGlnCysLeuArgArgLysGln SerSerLysHisAlaLeuGlyTyrThrLeu 360
361 HisProProSerGlnAlaValGluGlyGln HisLysAspMetValArgIleProValGly 380

FIG.6A

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381 SerArgGluThrPheTyrArgIleSerLys ThrAspGlyValCysGluTrpLysPhePhe 400
401 SerSerMetProArgGlySerAlaArgIle ThrValSerLysAspGlnSerSerCysThr 420
421 ThrAlaArgValArgSerLysSerPheLeu GlnValCysCysCysValGlyProSerThr 440
441 ProSerLeuAspLysAsnHisGlnValPro ThrIleLysValHisThrIleSerLeuSer 460
461 GluAsnGlyGluGluValEnd 467

FIG.6B

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1	AAT TCC CTC CTA GAA GCT GGA GAG AGC AGG AGC CTT CGG TGG GGC	45
46	AGC TCA AAA TGT AGG TAA CTG CGG GCC AGG AGC AGC GCC CAG ATG	90
91	CCA TCG GTC CCT GCC TTT GAG CGT CGA CGG CTG ATC TTT TGG TTT	135
136	GAG GGA GAG ACT GGC GCT GGA GTT TTG AAT TCC GAA TCA TGT GCA	180
181	GAA TCG TGA ATC TTC CCC CAG CCA GGA CGA ATA AGA CAG CGC GGA	225
226	AAA GCA GAT TCT CGT AAT TCT GGA ATT GCA TGT TGC AAG GAG TCT	270
271	CCT GGA TCT TCG CAC CCA GCT TCG GGT ACG GGA GGG AGT CCG GGT	315
316	CCC GGC TAG GCC AGC CCG CAG GTG GAG AGG GTC CCC GGC AGC CCC	360
361	GCG CGC CCC TGG CCA TGT CTT TAA TGC CCT GCC CCT TCA TGT GGC	405
406	CTT CTG AGG GTT CCC AGG GCT GGC CAG GGT TGT CTC CCA CCC GCG	450
451	CGC GCC GTC TCA CCC CCA GCC AAA CCC ACC TGG CAG GGC TCC CTC	495
496	CAG AAG AGA CCT TTT GAT TCC CGG CTC CCG CGC TCC CGC CTC CGC	540
541	GCC AGC CCG GGA GGT GGC CCT GGA CAG CCG GAC CTC GCC CGG CCC	585
586	CGG CTG NGG ACC ATG GTG TTT CTC TCG GGA AAT GCT TCC GAC AGC	630
1	Met Val Phe Leu Ser Gly Asn Ala Ser Asp Ser	11
631	TCC AAC TGC ACC CAA CCG CCG GCA CCG GTG AAC ATT TCC AAG GCC	675
12	Ser Asn Cys Thr Gln Pro Pro Ala Pro Val Asn Ile Ser Lys Ala	26
676	ATT CTG CTC GGG GTG ATC TTG GGG GGC CTC ATT CTT TTC GGG GTG	720
27	Ile Leu Leu Gly Val Ile Leu Gly Gly Leu Ile Leu Phe Gly Val	41
721	CTG GGT AAC ATC CTA GTG ATC CTC TCC GTA GCC TGT CAC CGA CAC	765
42	Leu Gly Asn Ile Leu Val Ile Leu Ser Val Ala Cys His Arg His	56
766	CTG CAC TCA GTC ACG CAC TAC TAC ATC GTC AAC CTG GCG GTG GCC	810
57	Leu His Ser Val Thr His Tyr Tyr Ile Val Asn Leu Ala Val Ala	71

FIG.7A

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811	GAC CTC CTG CTC ACC TCC ACG GTG CTG CCC TTC TCC GCC ATC TTC	855
72	Asp Leu Leu Leu Thr Ser Thr Val Leu Pro Phe Ser Ala Ile Phe	86
856	GAG GTC CTA GGC TAC TGG GCC TTC GGC AGG GTC TTC TGC AAC ATC	900
87	Glu Val Leu Gly Tyr Trp Ala Phe Gly Arg Val Phe Cys Asn Ile	101
901	TGG GCG GCA GTG GAT GTG CTG TGC TGC ACC GCG TCC ATC ATG GGC	945
102	Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala Ser Ile Met Gly	116
946	CTC TGC ATC ATC TCC ATC GAC CGC TAC ATC GGC GTG AGC TAC CCG	990
117	Leu Cys Ile Ile Ser Ile Asp Arg Tyr Ile Gly Val Ser Tyr Pro	131
991	CTG CGC TAC CCA ACC ATC GTC ACC CAG AGG AGG GGT CTC ATG GCT	1035
132	Leu Arg Tyr Pro Thr Ile Val Thr Gln Arg Arg Gly Leu Met Ala	146
1036	CTG CTC TGC GTC TGG GCA CTC TCC CTG GTC ATA TCC ATT GGA CCC	1080
147	Leu Leu Cys Val Trp Ala Leu Ser Leu Val Ile Ser Ile Gly Pro	161
1081	CTC TTC GGC TGG AGG CAG CCG GCC CCC GAG GAC GAG ACC ATC TGC	1125
162	Leu Phe Gly Trp Arg Gln Pro Ala Pro Glu Asp Glu Thr Ile Cys	176
1126	CAG ATC AAC GAG GAG CCG GGC TAC GTG CTC TTC TCG GCT CTG GGC	1170
177	Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu Gly	191
1171	TCC TTC TAC CTG CCT CTG GCC ATC ATC CTG GTC ATG TAC TGC CGC	1215
192	Ser Phe Tyr Leu Pro Leu Ala Ile Ile Leu Val Met Tyr Cys Arg	206
1216	GTC TAC GTG GTG GCC AAG AGG GAG AGC CGG GGC CTC AAG TCT GGC	1260
207	Val Tyr Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly	221
1261	CTC AAG ACC GAC AAG TCG GAC TCG GAG CAA GTG ACG CTC CGC ATC	1305
222	Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile	236
1306	CAT CGG AAA AAC GCC CCG GCA GGA GGC AGC GGG ATG GCC AGC GCC	1350
237	His Arg Lys Asn Ala Pro Ala Gly Gly Ser Gly Met Ala Ser Ala	251
1351	AAG ACC AAG ACG CAC TTC TCA GTG AGG CTC CTC AAG TTC TCC CGG	1395
252	Lys Thr Lys Thr His Phe Ser Val Arg Leu Leu Lys Phe Ser Arg	266
1396	GAG AAG AAA GCG GCC AAA ACG CTG GGC ATC GTG GTC GGC TGC TTC	1440
267	Glu Lys Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly Cys Phe	281

FIG.7B

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1441 GTC CTC TGC TGG CTG CCT TTT TTC TTA GTC ATG CCC ATT GGG TCT 1485
 282 Val Leu Cys Trp Leu Pro Phe Phe Leu Val Met Pro Ile Gly Ser 296

 1486 TTC TTC CCT GAT TTC AAG CCC TCT GAA ACA GTT TTT AAA ATA GTA 1530
 297 Phe Phe Pro Asp Phe Lys Pro Ser Glu Thr Val Phe Lys Ile Val 311

 1531 TTT TGG CTC GGA TAT CTA AAC AGC TGC ATC AAC CCC ATC ATA TAC 1575
 312 Phe Trp Leu Gly Tyr Leu Asn Ser Cys Ile Asn Pro Ile Ile Tyr 326

 1576 CCA TGC TCC AGC CAA GAG TTC AAA AAG GCC TTT CAG AAT GTC TTG 1620
 327 Pro Cys Ser Ser Gln Glu Phe Lys Lys Ala Phe Gln Asn Val Leu 341

 1621 AGA ATC CAG TGT CTC CGC AGA AAG CAG TCT TCC AAA CAT GCC CTG 1665
 342 Arg Ile Gln Cys Leu Arg Arg Lys Gln Ser Ser Lys His Ala Leu 356

 1666 GGC TAC ACC CTG CAC CCG CCC AGC CAG GCC GTG GAA GGG CAA CAC 1710
 357 Gly Tyr Thr Leu His Pro Pro Ser Gln Ala Val Glu Gly Gln His 371

 1711 AAG GAC ATG GTG CGC ATC CCC GTG GGA TCA AGA GAG ACC TTC TAC 1755
 372 Lys Asp Met Val Arg Ile Pro Val Gly Ser Arg Glu Thr Phe Tyr 386

 1756 AGG ATC TCC AAG ACG GAT GGC GTT TGT GAA TGG AAA TTT TTC TCT 1800
 387 Arg Ile Ser Lys Thr Asp Gly Val Cys Glu Trp Lys Phe Phe Ser 401

 1801 TCC ATG CCC CGT GGA TCT GCC AGG ATT ACA GTG TCC AAA GAC CAA 1845
 402 Ser Met Pro Arg Gly Ser Ala Arg Ile Thr Val Ser Lys Asp Gln 416

 1846 TCC TCC TGT ACC ACA GCC CGG GTG AGA AGT AAA AGC TTT TTG CAG 1890
 417 Ser Ser Cys Thr Thr Ala Arg Val Arg Ser Lys Ser Phe Leu Gln 431

 1891 GTC TGC TGC TGT GTA GGG CCC TCA ACC CCC AGC CTT GAC AAG AAC 1935
 432 Val Cys Cys Cys Val Gly Pro Ser Thr Pro Ser Leu Asp Lys Asn 446

 1936 CAT CAA GTT CCA ACC ATT AAG GTC CAC ACC ATC TCC CTC AGT GAG 1980
 447 His Gln Val Pro Thr Ile Lys Val His Thr Ile Ser Leu Ser Glu 461

 1981 AAC GGC GAA GAG GTT TAA GAA TTC 2004
 462 Asn Gly Glu Glu Val End 668

FIG.7C

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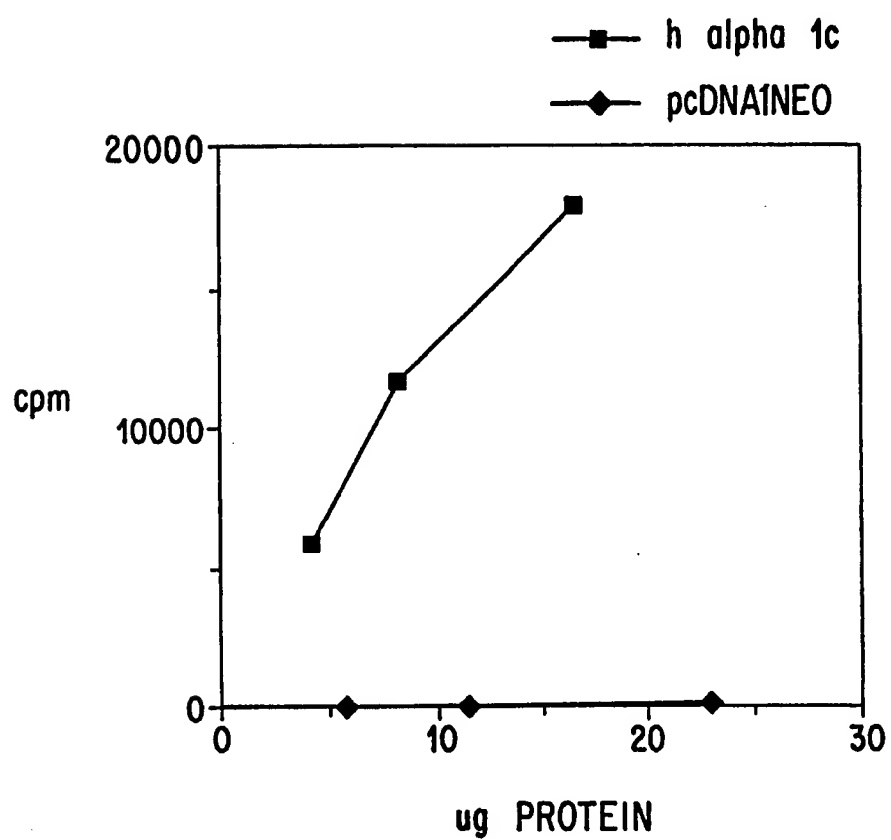


FIG. 8

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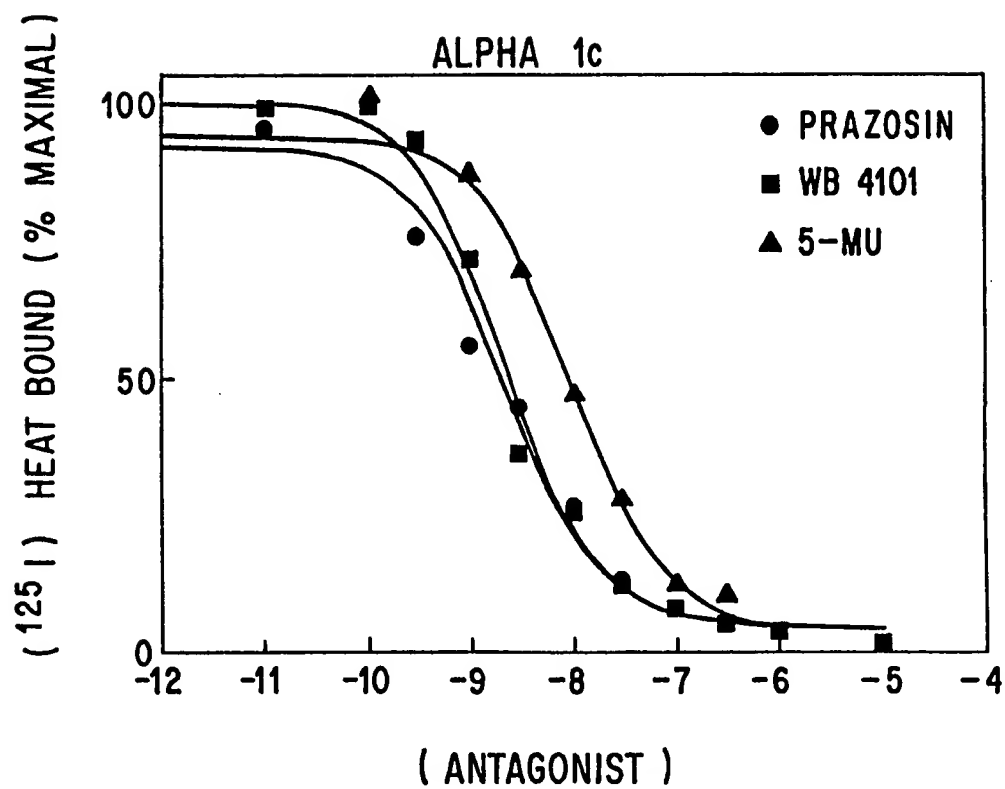


FIG. 9

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1	CCCGTGCAGG	GGCCCTACGG	ACACCACCAG	GGCTACGACC	CAGAGCAGGG	CCAGGATGGC	60
61	GGCCGCCTTG	CGCTCGGTCA	TGATGGCTGG	GTACTTGAGT	GAGTGGCGCA	CGCCACGTA	120
121	CCGGTCCACG	GAGATGGTGC	AGAGGCTGAG	GATGGAGGCC	GTGCAGCACA	GCACGTCCAC	180
181	GGCGGCCGTC	GGGGGACTGG	TGGTGAGCGC	GCAGGGCGTG	GGCGTGGGCG	TCTTCCTGGC	240
241	AGCCTTCATC	CTTATGGCCG	TGGCAGGTAA	CCTGCTTGTC	ATCCTCTCAG	TGGCCTGCAA	300
301	CGCCACCTG	CAGACCGTCA	CCAACTATTT	CATCGTGAAC	CTGGCCGTGG	CCGACCTGCT	360
361	GCTGAGCGCC	ACCGTACTGC	CCTTCTCGGC	CACCATGGAG	GTTCTGGGCT	TCTGGGCCTT	420
421	TGGCCGCGCC	TTCTGCGACG	TATGGGCCGC	CGTGGACGTG	CTGTGCTGCA	CGGCCTCCAT	480
481	CCTCAGCCTC	TGCACCATCT	CCGTGGACCG	GTACGTGGGC	GTGCGCCACT	CACTCAAGTA	540
541	CCCAGCCATC	ATGACCGAGC	GCAAGGCGGC	CGCCATCCTG	GCCCTGCTCT	GGGTGCTAGC	600
601	CCTGGTGGTG	TCCGTAGGGC	CCCTGCTGGG	CTGGAAGGAG	CCCGTGCCCC	CTGACGAGCG	660
661	CTTCTCGGGT	ATCACCGAGG	AGGCGGGCTA	CGCTGTCTTC	TCCTCCGTGT	GCTCCTTCTA	720
721	CCTGCCCATG	GCGGTCATCG	TGGTCATGTA	CTGCCGCGTG	TACGTGGTCG	CGCGCAGCAC	780
781	CACGCGCAGC	CTCGAGGCAG	GCGTCAAGCG	CGAGCGAGGC	AAGGCCTCCG	AGGTGGTGCT	840

FIG.10A

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841 GCGCATCCAC TGTCGCGGCG CGGCCACGGG CGCCGACGGG GCGCACGGCA TCGCAGCGC 900

901 CAAGGGCCAC ACCTCCGCA GCTCGCTCTC CGTGCGCCTG CTCAAGTTCT CCCGTGAGAA 960

961 GAAAGCGGCC AAGACTCTGG CCATCGTCGT GGGTGTCTTC GTGCTCTGCT GGTCCCTTT 1020

1021 CTTCTTTGTC CTGCCGCTCG GTCCTTGTT CCCGCAGCTG AAGCCATCGG AGGGCGTCTT 1080

1081 CAAGGTCATC TTCTGGCTCG GCTACTTCAA CAGCTGCGTG AACCCGCTCA TCTACCCCTG 1140

1141 TTCCAGCCGC GAGTCAAGC GCGCCTTCCT CCGTCTCCTG CGCTGCCAGT GCCGTCGTG 1200

1201 CCGGCGCCGC CGCCCTCTCT GCGTGTCTA CGGCCACCAC TGGCGGGCCT CCACCAGCGG 1260

1261 CCTGCGCCAG GACTGCGCCC CGAGTTCGGG CGACGCGCCC CCCGAGCGC CGCTGGCCCT 1320

1321 CACCGCGCTC CCCGACCCCG ACCCGAACC CCCAGGCAG CCCGAGATGC AGGCTCCGT 1380

1381 CGCCAGCCGT CGAAGCCACC CAGCGCCTTC CGCGAGTGA GGCTGCTGGG GCCGTTCCG 1440

1441 AGACCCACGA CCCAGCTGCG CGCCAAAGTC GCCAGCCTGT CGCACAAGAT CGCCGCCGGG 1500

1501 GGCGCGCAGC GCGCAGAGGC AGCGTGCGCC CAGCGCTCAG AGGTGAGGC TGTGTCCCTA 1560

1561 GGCGTCCAC ACGAGGTGGC CGAGGGCGCC ACCTGCCAGG CCTACGAATT GCGCGACTAC 1620

1621 A 1621

FIG.10B

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1 MetAlaAlaAlaLeuArgSerValMetMet AlaGlyTyrLeuSerGluTrpArgThrPro 20
21 ThrTyrArgSerThrGluMetValGlnArg LeuArgMetGluAlaValGlnHisSerThr 40
41 SerThrAlaAlaValGlyGlyLeuValVal SerAlaGlnGlyValGlyValGlyValPhe 60
61 LeuAlaAlaPheIleLeuMetAlaValAla GlyAsnLeuLeuValIleLeuSerValAla 80
81 CysAsnArgHisLeuGlnThrValThrAsn TyrPheIleValAsnLeuAlaValAlaAsp 100
101 LeuLeuLeuSerAlaThrValLeuProPhe SerAlaThrMetGluValLeuGlyPheTrp 120
121 AlaPheGlyArgAlaPheCysAspValTrp AlaAlaValAspValLeuCysCysThrAla 140
141 SerIleLeuSerLeuCysThrIleSerVal AspArgTyrValGlyValArgHisSerLeu 160
161 LysTyrProAlaIleMetThrGluArgLys AlaAlaAlaIleLeuAlaLeuLeuTrpVal 180
181 ValAlaLeuValValSerValGlyProLeu LeuGlyTrpLysGluProValProProAsp 200
201 GluArgPheCysGlyIleThrGluGluAla GlyTyrAlaValPheSerSerValCysSer 220
221 PheTyrLeuProMetAlaValIleValVal MetTyrCysArgValTyrValValAlaArg 240
241 SerThrThrArgSerLeuGluAlaGlyVal LysArgGluArgGlyLysAlaSerGluVal 260
261 ValLeuArgIleHisCysArgGlyAlaAla ThrGlyAlaAspGlyAlaHisGlyMetArg 280
281 SerAlaLysGlyHisThrPheArgSerSer LeuSerValArgLeuLeuLysPheSerArg 300
301 GluLysLysAlaAlaLysThrLeuAlaIle ValValGlyValPheValLeuCysTrpPhe 320
321 ProPhePhePheValLeuProLeuGlySer LeuPheProGlnLeuLysProSerGluGly 340
341 ValPheLysValIlePheTrpLeuGlyTyr PheAsnSerCysValAsnProLeuIleTyr 360
361 ProCysSerSerArgGluPheLysArgAla PheLeuArgLeuLeuArgCysGlnCysArg 380
381 ArgArgArgArgArgArgProLeuTrpArg ValTyrGlyHisHisTrpArgAlaSerThr 400

FIG. 11A

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401 SerGlyLeuArgGlnAspCysAlaProSer SerGlyAspAlaProProGlyAlaProLeu 420
421 AlaLeuThrAlaLeuProAspProAspPro GluProProGlyThrProGluMetGlnAla 440
441 ProValAlaSerArgArgSerHisProAla ProSerAlaSerGlyGlyCysTrpGlyArg 460
461 SerGlyAspProArgProSerCysAlaPro LysSerProAlaCysArgThrArgSerPro 480
481 ProGlyAlaArgSerAlaGlnArgGlnArg AlaProSerAlaGlnArgTrpArgLeuCys 500
501 Pro

FIG.11B

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TCTAGACCATGAATCCCGACCTGGACACCGGCCACAACACATCAGCACCTGCCCCACTGGGGAGAGTTG
AAAAATGCCAACTTCACTGGCCCCAACCAGACCTCGAGCAACTCCACACTGCCCCAGCTG
GACATCACCAGGGCCATCTCTGTGGGCCTGGTGTGGGGCCTTCATCCTCTTTGCCATC
GTGGGCAACATCCTAGTCATCTTGTCTGTGGCCTGCAACCGGCACCTGCGGACGCCCACC
AACTACTTCATTGTCAACCTGGCCATGGCCGACCTGCTGTTGAGCTTCACCGTCCTGCCC
TTCTCAGCGGCCCTAGAGGTGCTCGGCTACTGGGTGCTGGGGCGGATCTTCTGTGACATC
TGGGCAGCCGTGGATGTCTGTGCTGCACAGCGTCCATTCTGAGCCTGTGCGCCATCTCC
ATCGATCGCTACATCGGGGTGCGCTACTCTCTGCAGTATCCCACGCTGGTCACCCGGAGG
AAGGCCATCTTGGCCCTGCTCAGTGTCTGGGTCTTGTCCACCGTCATCTCCATCGGGCCT
CTCCTTGGGTGGAAGGAGCCGGCACCCAACGATGACAAGGAGTGCGGGGTACCGAAGAA
CCCTTCTATGCCCTCTTCTCCTCTCTGGGCTCCTTCTACATCCCTCTGGCGGTCAATTCTA
GTCATGTACTGCCGTGTCTATATAGTGGCCAAGAGAACCACCAAGAACCTAGAGGCAGGA
GTCATGAAGGAGATGTCCAACCTCCAAGGAGCTGACCCTGAGGATCCATTCCAAGAACTTT
CACGAGGACACCCTTAGCAGTACCAAGGCCAAGGGCCACAACCCAGGAGTTCCATAGCT
GTCAAACTTTTTAAGTTCTCCAGGAAAAGAAAGCAGCTAAGACGTTGGGCATTGTGGTC
GGTATGTGAATTC

FIG.12

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1 AAGAGAACCA CCAAGAACCT AGAGGCAGGA GTCATGAAGG AGATGTCCAA CTCCAAGGAG 60
61 CTGACCCTGA GGATCCATTC CAAGAACTTT CACGAGGACA CCCTTAGCAG TACCAAGGCC 120
121 AAGGGCCACA ACCCCAGGAG TTCCATAGCT GTCAAACITT TTAAGTTCTC CAGGGAAAAG 180
181 AAAGCAGCTA AGACGTGGG CATTGTGGTC GGTATGTTCA TCTTGTGCTG GCTACCCTTC 240
241 TTCATCGCTC TACCGCTTGG CTCCTTGTTT TCCACCCTGA AGCCCCCGA CGCCGTGTTT 300
301 AAGGTGGTGT TCTGGCTGGG CTACTTCAAC AGCTGCCTCA ACCCATCAT CTACCATGC 360
361 TCCAGCAAGG AGTTCAAGCG CGCTTTCGT 389

FIG.13

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GAATTCATGA TTCAAGGTGG TGTTCTGGCT GGGCTACTTC AACAGCTGCC TCAATCCCAT CATCTACCCG
TGCTCCAGCA AGGAGTTCAA GCGCGCCTTC ATGCGTATCC TTGGGTGCCA GTGCCGCGGT GGCCGCCGCC
GCCGCCGCCG TCGCCGTCTA GCGCGTGCG CTTACACCTA CCGGCCGTGG ACCCGCGGCG GCTCGCTGGA
GAGATCACAG TCGCGGAAGG ACTCTCTGGA TGACAGCGGC AGCTGCATGA GCGGCCAGAA GAGGACCCTG
CCCTCGGCGT CGCCAGCCC GGGCTACCTG GGTGAGGAA CGCAGCCACC CGTGGAGCTG TGCGCCTTCC
CCGAGTGAA ACCCGGGCG CTGCTCAGCT TGCCAGAGCC TCCTGGCCGC CGCGGCCGTC TCGACTCTGG
GCCACTCTTC ACCTCAAGC TCCTGGGCGA TCCTGAGAGC CCGGAACCG AAGCGACAGC CAGCAACGGG
GGCTGCGACA CCACGACCGA CCTGGCCAAC GGGCAGCCCG GCTTCAAGAG CAACATGCCC CTGGGCCCGG
GCCACTTTTA AAAGCCGAATTC

FIG.14

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1 TCTAGACCAT GAATCCCGAC CTGGACACCG GCCACAACAC ATCAGCACCT
51 GCCCACTGGG GAGAGTTGAA AAATGCCAAC TTCACTGGCC CCAACCAGAC
101 CTCGAGCAAC TCCACACTGC CCCAGCTGGA CATCACCAGG GCCATCTCTG
151 TGGGCTGGT GCTGGGCGCC TTCATCCTCT TTGCCATCGT GGGCAACATC
201 CTAGTCATCT TGTCTGTGGC CTGCAACCGG CACCTGCGGA CGCCACCAA
251 CTACTTCATT GTCAACCTGG CCATGGCCGA CCTGCTGTTG AGCTTCACCG
301 TCCTGCCCTT CTCAGCGGCC CTAGAGGTGC TCGGCTACTG GGTGCTGGGG
351 CGGATCTTCT GTGACATCTG GGCAGCCGTG GATGTCCTGT GCTGCACAGC
401 GTCCATTCTG AGCCTGTGCG CCATCTCCAT CGATCGCTAC ATCGGGGTGC
451 GCTACTCTCT GCAGTATCCC ACGCTGGTCA CCCGGAGGAA GGCCATCTTG
501 GCCCTGCTCA GTGTCTGGGT CTTGTCCACC GTCATCTCCA TCGGGCTCT
551 CCTTGGGTGG AAGGAGCCGG CACCCAACGA TGACAAGGAG TGCGGGGTCA
601 CCGAAGAACC CTCTATGCC CTCTTCTCCT CTCTGGGCTC CTTCTACATC
651 CCTCTGGCGG TCATTCTAGT CATGTACTGC CGTGTCTATA TAGTGGCCAA
701 GAGAACCACC AGAACCTAG AGGCAGGAGT CATGAAGGAG ATGTCCAAC
751 CCAAGGAGCT GACCCTGAGG ATCCATTCCA AGAACTTTCA CGAGGACACC
801 CTTAGCAGTA CCAAGGCCAA GGGCCACAAC CCCAGGAGTT CCATAGCTGT
851 CAAACTTTTT AAGTTCTCCA GGGAAAAGAA AGCAGCTAAG ACGTTGGGCA
901 TTGTGGTCGG TATGTTTCATC TTGTGCTGGC TACCCTTCTT CATCGCTCTA
951 CCGCTTGGCT CCTTGTCTC CACCCTGAAG CCCCCGACG CCGTGTTCAA

FIG.15A

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1001 GGTGGTGTTC TGGCTGGGCT ACTTCAACAG CTGCCTCAAC CCCATCATCT
1051 ACCCATGCTC CAGCAAGGAG TTCAAGCGCG CCTTCATGCG TATCCTTGGG
1101 TGCCAGTGCC GCGGTGGCCG CCGCCGCCCG CGCCGTCGCC GTCTAGGCGG
1151 GTGCGCTTAC ACCTACCGGC CGTGGACCCG CGGCGGCTCG CTGGAGAGAT
1201 CACAGTCGCG GAAGGACTCT CTGGATGACA GCGGCAGCTG CATGAGCGGC
1251 CAGAAGAGGA CCCTGCCCTC GCGTCCGCC AGCCCGGGCT ACCTGGGTGG
1301 AGGAACGCAG CCACCCGTGG AGCTGTGCGC CTTCCCCGAG TGGAAACCCG
1351 GGGCGCTGCT CAGCTTGCCA GAGCTCCTG GCCGCCGCGG CCGTCTCGAC
1401 TCTGGGCCAC TCTTACCTT CAAGCTCCTG GCGATCCTG AGAGCCCGGG
1451 AACCGAAGCG ACAGCCAGCA ACGGGGGCTG CGACACCAG ACCGACCTGG
1501 CCAACGGGCA GCCCGGCTT AAGAGCAACA TGCCCTGGG CCCGGGCCAC
1551 TTTTAAAGC CGAATTC

FIG.15B

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1 MetAsnProAspLeuAspThrGlyHisAsn ThrSerAlaProAlaHisTrpGlyGluLeu 20
21 LysAsnAlaAsnPheThrGlyProAsnGln ThrSerSerAsnSerThrLeuProGlnLeu 40
41 AspIleThrArgAlaIleSerValGlyLeu ValLeuGlyAlaPheIleLeuPheAlaIle 60
61 ValGlyAsnIleLeuValIleLeuSerVal AlaCysAsnArgHisLeuArgThrProThr 80
81 AsnTyrPheIleValAsnLeuAlaMetAla AspLeuLeuLeuSerPheThrValLeuPro 100
101 PheSerAlaAlaLeuGluValLeuGlyTyr TrpValLeuGlyArgIlePheCysAspIle 120
121 TrpAlaAlaValAspValLeuCysCysThr AlaSerIleLeuSerLeuCysAlaIleSer 140
141 IleAspArgTyrIleGlyValArgTyrSer LeuGlnTyrProThrLeuValThrArgArg 160
161 LysAlaIleLeuAlaLeuLeuSerValTrp ValLeuSerThrValIleSerIleGlyPro 180
181 LeuLeuGlyTrpLysGluProAlaProAsn AspAspLysGluCysGlyValThrGluGlu 200
201 ProPheTyrAlaLeuPheSerSerLeuGly SerPheTyrIleProLeuAlaValIleLeu 220
221 ValMetTyrCysArgValTyrIleValAla LysArgThrThrLysAsnLeuGluAlaGly 240
241 ValMetLysGluMetSerAsnSerLysGlu LeuThrLeuArgIleHisSerLysAsnPhe 260
261 HisGluAspThrLeuSerSerThrLysAla LysGlyHisAsnProArgSerSerIleAla 280
281 ValLysLeuPheLysPheSerArgGluLys LysAlaAlaLysThrLeuGlyIleValVal 300
301 GlyMetPheIleLeuCysTrpLeuProPhe PheIleAlaLeuProLeuGlySerLeuPhe 320
321 SerThrLeuLysProProAspAlaValPhe LysValValPheTrpLeuGlyTyrPheAsn 340
341 SerCysLeuAsnProIleIleTyrProCys SerSerLysGluPheLysArgAlaPheMet 360
361 ArgIleLeuGlyCysGlnCysArgGlyGly ArgArgArgArgArgArgArgArgLeuGly 380
381 AlaCysAlaTyrThrTyrArgProTrpThr ArgGlyGlySerLeuGluArgSerGlnSer 400

FIG.16A

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401 ArgLysAspSerLeuAspAspSerGlySer CysMetSerGlyGlnLysArgThrLeuPro 420
421 SerAlaSerProSerProGlyTyrLeuGly ArgGlyThrGlnProProValGluLeuCys 440
441 AlaPheProGluTrpLysProGlyAlaLeu LeuSerLeuProGluProProGlyArgArg 460
461 GlyArgLeuAspSerGlyProLeuPheThr PheLysLeuLeuGlyAspProGluSerPro 480
481 GlyThrGluAlaThrAlaSerAsnGlyGly CysAspThrThrThrAspLeuAlaAsnGly 500
501 GlnProGlyPheLysSerAsnMetProLeu GlyProGlyHisPhe

FIG.16B

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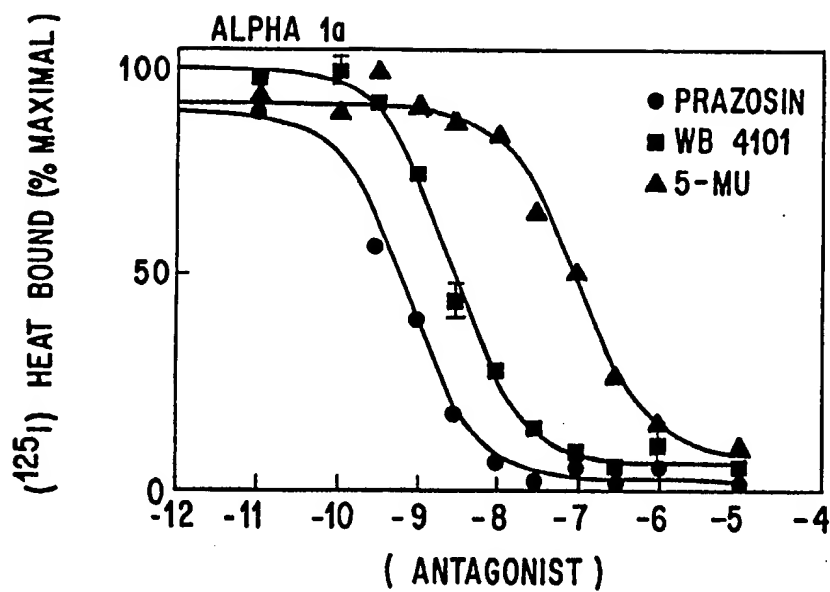


FIG. 17A

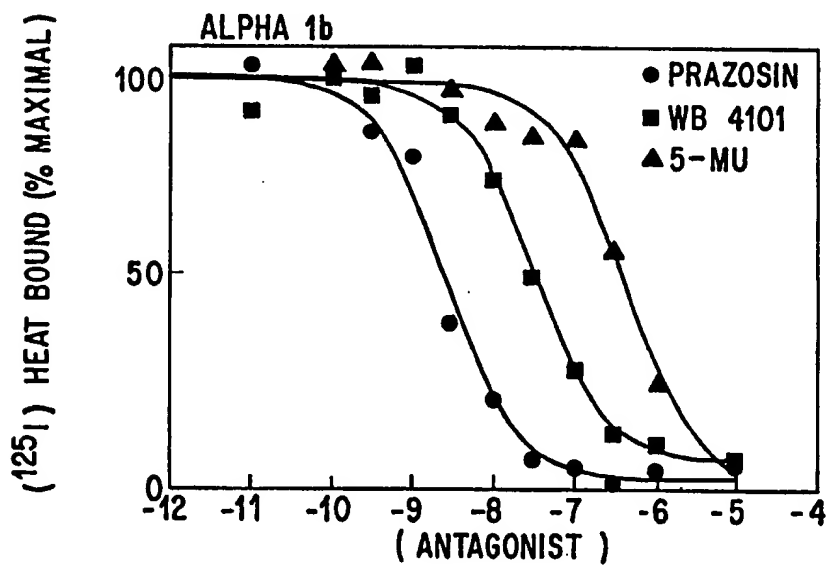


FIG. 17B

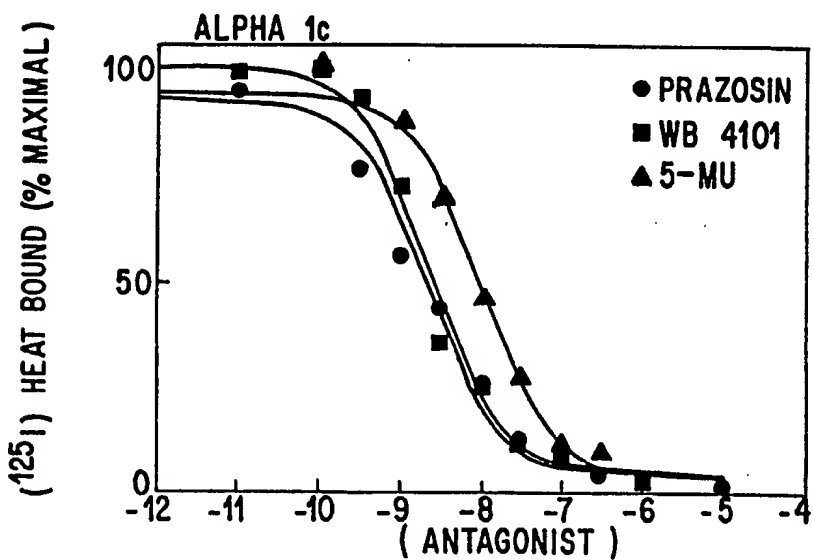


FIG. 17C

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1 GAATTCCTC CTAGAAGCTG GAGAGAGCAG GAGCCTTCGG TGGGCAGCT
51 CAAATGTAG GTAAGTGGG GCCAGGAGCA GCGCCAGAT GCCATCGGT
101 CCTGCCTTTG AGCGTCGACG GCTGATCTTT TGGTTTGAGG GAGAGACTGG
151 CGCTGGAGTT TTGAATTCCG AATCATGTGC AGAATCGTA ATCTTCCCC
201 AGCCAGGACG AATAAGACAG CGCGAAAAG CAGATTCTCG TAATTCTGGA
251 ATTGCATGTT GCAAGGAGTC TCCTGGATCT TCGCACCAG CTTGGGTAC
301 GGGAGGGAGT CCGGTCCCG GCTAGGCCAG CCCGAGGTG GAGAGGTCC
351 CCGGCAGCCC CGCGCGCCC TGGCCATGTC TTTAATGCC TGCCCTTCA
401 TGTGGCCTTC TGAGGGTTCC CAGGGCTGGC CAGGGTTGTC TCCACCCGC
451 GCGCGCGTC TCACCCCGAG CCAAACCCAC CTGGCAGGC TCCCTCCAGA
501 AGAGACCTTT TGATTCCCG CTCCCGCGCT CCCGCCTCCG CGCCAGCCCG
551 GGAGGTGGCC CTGGACAGCC GGACCTCGCC CGGCCCCGGC TGNGGACCAT
601 GGTGTTTCTC TCGGAAATG CTTCCGACAG CTCCAACTGC ACCCAACCGC
651 CGGCACCGT GAACATTTCC AAGGCCATTG TGCTCGGGT GATCTTGGG
701 GGCTCATTC TTTTCGGGT GCTGGTAAC ATCCTAGTA TCCTCTCCGT
751 AGCCTGTCAC CGACACCTGC ACTCAGTCAC GCACTACTAC ATCGTCAACC
801 TGGCGTGCC CGACCTCTG CTCACCTCA CGGTGCTGCC CTTCTCCGC
851 ATCTTCGAGG TCCTAGGCTA CTGGCCTTC GGCAGGTCT TCTGCAACAT
901 CTGGCGGCA GTGGATGTG TGTGCTGCAC CGGTCCATC ATGGGCCTCT
951 GCATCATCTC CATCGACCG TACATCGCG TGAGTACCC GCTGCGCTAC

FIG.18A

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1001 CCAACCATCG TCACCCAGAG GAGGGTCTC ATGGCTCTGC TCTGCGTCTG
1051 GGCACCTCTCC CTGGTCATAT CCATTGGACC CCTCTTCGGC TGGAGGCAGC
1101 CGGCCCCCGA GGACGAGACC ATCTGCCAGA TCAACGAGGA GCCGGGCTAC
1151 GTGCTCTTCT CGGCTCTGGG CTCCTTCTAC CTGCCTCTGG CCATCATCCT
1201 GGTACGTAC TGCCGCGTCT ACGTGGTGGC CAAGAGGGAG AGCCGGGGCC
1251 TCAAGTCTGG CCTCAAGACC GACAAGTCGG ACTCGGAGCA AGTGACGCTC
1301 CGCATCCATC GGAAAAACGC CCCGGCAGGA GGCAGCGGA TGGCCAGCGC
1351 CAAGACCAAG ACGCACTTCT CAGTGAGGCT CCTCAAGTTC TCCCGGGAGA
1401 AGAAAGCGGC CAAAACGCTG GGCATCGTGG TCGGCTGCTT CGTCCTCTGC
1451 TGGCTGCCTT TTTTCTTAGT CATGCCATT GGGTCTTCT TCCCTGATT
1501 CAAGCCCTCT GAAACAGTTT TTAAATAGT ATTTTGGCTC GGATATCTAA
1551 ACAGCTGCAT CAACCCCATC ATATACCAT GCTCCAGCCA AGAGTTCAAA
1601 AAGGCCTTTC AGAATGTCTT GAGAATCCAG TGTCTCCGA GAAAGCAGTC
1651 TTCAAACAT GCCCTGGGCT ACACCCTGCA CCCGCCAGC CAGGCCGTGG
1701 AAGGGCAACA CAAGGACATG GTGCGCATCC CCGTGGGATC AAGAGAGACC
1751 TTCTACAGGA TCTCAAGAC GGATGGCGTT TGTGAATGA AATTTTCTC
1801 TTCCATGCCC CGTGGATCTG CCAGGATTAC AGTGTCAAA GACCAATCCT
1851 CCTGTACCAC AGCCCGGGTG AGAAGTAAA GCTTTTTCGA GGTCTGCTGC
1901 TGTGTAGGGC CCTCAACCCC CAGCCTTGAC AAGAACCATC AAGTTCCAAC
1951 CATTAGGTC CACACCATCT CCCTCAGT

FIG.18B

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1	AAT TCC CTC CTA GAA GCT GGA GAG AGC AGG AGC CTT CGG TGG GGC	45
46	AGC TCA AAA TGT AGG TAA CTG CGG GCC AGG AGC AGC GCC CAG ATG	90
91	CCA TCG GTC CCT GCC TTT GAG CGT CGA CGG CTG ATC TTT TGG TTT	135
136	GAG GGA GAG ACT GGC GCT GGA GTT TTG AAT TCC GAA TCA TGT GCA	180
181	GAA TCG TGA ATC TTC CCC CAG CCA GGA CGA ATA AGA CAG CGC GGA	225
226	AAA GCA GAT TCT CGT AAT TCT GGA ATT GCA TGT TGC AAG GAG TCT	270
271	CCT GGA TCT TCG CAC CCA GCT TCG GGT ACG GGA GGG AGT CCG GGT	315
316	CCC GGC TAG GCC AGC CCG CAG GTG GAG AGG GTC CCC GGC AGC CCC	360
361	GCG CGC CCC TGG CCA TGT CTT TAA TGC CCT GCC CCT TCA TGT GGC	405
406	CTT CTG AGG GTT CCC AGG GCT GGC CAG GGT TGT CTC CCA CCC GCG	450
451	CGC GCC GTC TCA CCC CCA GCC AAA CCC ACC TGG CAG GGC TCC CTC	495
496	CAG AAG AGA CCT TTT GAT TCC CGG CTC CCG CGC TCC CGC CTC CGC	540
541	GCC AGC CCG GGA GGT GGC CCT GGA CAG CCG GAC CTC GCC CGG CCC	585
586	CGG CTG NGG ACC ATG GTG TTT CTC TCG GGA AAT GCT TCC GAC AGC	630
631	TCC AAC TGC ACC CAA CCG CCG GCA CCG GTG AAC ATT TCC AAG GCC	675
676	ATT CTG CTC GGG GTG ATC TTG GGG GGC CTC ATT CTT TTC GGG GTG	720
721	CTG GGT AAC ATC CTA GTG ATC CTC TCC GTA GCC TGT CAC CGA CAC	765
766	CTG CAC TCA GTC ACG CAC TAC TAC ATC GTC AAC CTG GCG GTG GCC	810
811	GAC CTC CTG CTC ACC TCC ACG GTG CTG CCC TTC TCC GCC ATC TTC	855
856	GAG GTC CTA GGC TAC TGG GCC TTC GGC AGG GTC TTC TGC AAC ATC	900

FIG.19A

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901 TGG GCG GCA GTG GAT GTG CTG TGC TGC ACC GCG TCC ATC ATG GGC 945
946 CTC TGC ATC ATC TCC ATC GAC CGC TAC ATC GGC GTG AGC TAC CCC 990
991 CTG CGC TAC CCA ACC ATC GTC ACC CAG AGG AGG GGT CTC ATG GCT 1035
1036 CTG CTC TGC GTC TGG GCA CTC TCC CTG GTC ATA TCC ATT GGA CCC 1080
1081 CTC TTC GGC TGG AGG CAG CCG GCC CCC GAG GAC GAG ACC ATC TGC 1125
1126 CAG ATC AAC GAG GAG CCG GGC TAC GTG CTC TTC TCG GCT CTG GGC 1170
1171 TCC TTC TAC CTG CCT CTG GCC ATC ATC CTG GTC ATG TAC TGC CGC 1215
1216 GTC TAC GTG GTG GCC AAG AGG GAG AGC CGG GGC CTC AAG TCT GGC 1260
1261 CTC AAG ACC GAC AAG TCG GAC TCG GAG CAA GTG ACG CTC CGC ATC 1305
1306 CAT CGG AAA AAC GCC CCG GCA GGA GGC AGC GGG ATG GCC AGC GCC 1350
1351 AAG ACC AAG ACG CAC TTC TCA GTG AGG CTC CTC AAG TTC TCC CGG 1395
1396 GAG AAG AAA GCG GCC AAA ACG CTG GGC ATC GTG GTC GGC TGC TTC 1440
1441 GTC CTC TGC TGG CTG CCT TTT TTC TTA GTC ATG CCC ATT GGG TCT 1485
1486 TTC TTC CCT GAT TTC AAG CCC TCT GAA ACA GTT TTT AAA ATA GTA 1530
1531 TTT TGG CTC GGA TAT CTA AAC AGC TGC ATC AAC CCC ATC ATA TAC 1575
1576 CCA TGC TCC AGC CAA GAG TTC AAA AAG GCC TTT CAG AAT GTC TTG 1620
1621 AGA ATC CAG TGT CTC TGC AGA AAG CAG TCT TCC AAA CAT GCC CTG 1665
1666 GGC TAC ACC CTG CAC CCG CCC AGC CAG GCC GTG GAA GGG CAA CAC 1710
1711 AAG GAC ATG GTG CGC ATC CCC GTG GGA TCA AGA GAG ACC TTC TAC 1755

FIG.19B

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1756 AGG ATC TCC AAG ACG GAT GGC GTT TGT GAA TGG AAA TTT TTC TCT 1800
1801 TCC ATG CCC CGT GGA TCT GCC AGG ATT ACA GTG TCC AAA GAC CAA 1845
1846 TCC TCC TGT ACC ACA GCC CGG GTG AGA AGT AAA AGC TTT TTG CAG 1890
1891 GTC TGC TGC TGT GTA GGG CCC TCA ACC CCC AGC CTT GAC AAG AAC 1935
1936 CAT CAA GTT CCA ACC ATT AAG GTC CAC ACC ATC TCC CTC AGT GAG 1980
1981 AAC GGG GAG GAA GTC TAG 1998

FIG.19C

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1	Met	Val	Phe	Leu	Ser	Gly	Asn	Ala	Ser	Asp	Ser	Ser	Asn	Cys	Thr	
					Gln	Pro	Pro	Ala	Pro	Val	Asn	Ile	Ser	Lys	Ala	26
27	Ile	Leu	Leu	Gly	Val	Ile	Leu	Gly	Gly	Leu	Ile	Leu	Phe	Gly	Val	41
42	Leu	Gly	Asn	Ile	Leu	Val	Ile	Leu	Ser	Val	Ala	Cys	His	Arg	His	56
57	Leu	His	Ser	Val	Thr	His	Tyr	Tyr	Ile	Val	Asn	Leu	Ala	Val	Ala	71
72	Asp	Leu	Leu	Leu	Thr	Ser	Thr	Val	Leu	Pro	Phe	Ser	Ala	Ile	Phe	86
87	Glu	Val	Leu	Gly	Tyr	Trp	Ala	Phe	Gly	Arg	Val	Phe	Cys	Asn	Ile	101
102	Trp	Ala	Ala	Val	Asp	Val	Leu	Cys	Cys	Thr	Ala	Ser	Ile	Met	Gly	116
946	ctc	tgc	atc	atc	tcc	atc	gac	cgc	tac	atc	ggc	gtg	agc	tac	cCg	990
117	Leu	Cys	Ile	Ile	Ser	Ile	Asp	Arg	Tyr	Ile	Gly	Val	Ser	Tyr	Pro	131
132	Leu	Arg	Tyr	Pro	Thr	Ile	Val	Thr	Gln	Arg	Arg	Gly	Leu	Met	Ala	146
147	Leu	Leu	Cys	Val	Trp	Ala	Leu	Ser	Leu	Val	Ile	Ser	Ile	Gly	Pro	161
162	Leu	Phe	Gly	Trp	Arg	Gln	Pro	Ala	Pro	Glu	Asp	Glu	Thr	Ile	Cys	176
177	Gln	Ile	Asn	Glu	Glu	Pro	Gly	Tyr	Val	Leu	Phe	Ser	Ala	Leu	Gly	191
192	Ser	Phe	Tyr	Leu	Pro	Leu	Ala	Ile	Ile	Leu	Val	Met	Tyr	Cys	Arg	206
207	Val	Tyr	Val	Val	Ala	Lys	Arg	Glu	Ser	Arg	Gly	Leu	Lys	Ser	Gly	221
222	Leu	Lys	Thr	Asp	Lys	Ser	Asp	Ser	Glu	Gln	Val	Thr	Leu	Arg	Ile	236
237	His	Arg	Lys	Asn	Ala	Pro	Ala	Gly	Gly	Ser	Gly	Met	Ala	Ser	Ala	251
252	Lys	Thr	Lys	Thr	His	Phe	Ser	Val	Arg	Leu	Leu	Lys	Phe	Ser	Arg	266
267	Glu	Lys	Lys	Ala	Ala	Lys	Thr	Leu	Gly	Ile	Val	Val	Gly	Cys	Phe	281
282	Val	Leu	Cys	Trp	Leu	Pro	Phe	Phe	Leu	Val	Met	Pro	Ile	Gly	Ser	296
297	Phe	Phe	Pro	Asp	Phe	Lys	Pro	Ser	Glu	Thr	Val	Phe	Lys	Ile	Val	311
312	Phe	Trp	Leu	Gly	Tyr	Leu	Asn	Ser	Cys	Ile	Asn	Pro	Ile	Ile	Tyr	326
327	Pro	Cys	Ser	Ser	Gln	Glu	Phe	Lys	Lys	Ala	Phe	Gln	Asn	Val	Leu	341
342	Arg	Ile	Gln	Cys	Leu	CYS	Arg	Lys	Gln	Ser	Ser	Lys	His	Ala	Leu	356
357	Gly	Tyr	Thr	Leu	His	Pro	Pro	Ser	Gln	Ala	Val	Glu	Gly	Gln	His	371
372	Lys	Asp	Met	Val	Arg	Ile	Pro	Val	Gly	Ser	Arg	Glu	Thr	Phe	Tyr	386
387	Arg	Ile	Ser	Lys	Thr	Asp	Gly	Val	Cys	Glu	Trp	Lys	Phe	Phe	Ser	401
402	Ser	Met	Pro	Arg	Gly	Ser	Ala	Arg	Ile	Thr	Val	Ser	Lys	Asp	Gln	416
417	Ser	Ser	Cys	Thr	Thr	Ala	Arg	Val	Arg	Ser	Lys	Ser	Phe	Leu	Gln	431
432	Val	Cys	Cys	Cys	Val	Gly	Pro	Ser	Thr	Pro	Ser	Leu	Asp	Lys	Asn	446
447	His	Gln	Val	Pro	Thr	Ile	Lys	Val	His	Thr	Ile	Ser	Leu	Ser	Glu	461
462	Asn	Gly	Glu	Glu	Val	End										466

FIG.20

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1 AAT TCC CTC CTA GAA GCT GGA GAG AGC AGG AGC CTT CGG TGG GGC 45
 46 AGC TCA AAA TGT AGG TAA CTG CGG GCC AGG AGC AGC GCC CAG ATG 90
 91 CCA TCG GTC CCT GCC TTT GAG CGT CGA CGG CTG ATC TTT TGG TTT 135
 136 GAG GGA GAG ACT GGC GCT GGA GTT TTG AAT TCC GAA TCA TGT GCA 180
 181 GAA TCG TGA ATC TTC CCC CAG CCA GGA CGA ATA AGA CAG CGC GGA 225
 226 AAA GCA GAT TCT CGT AAT TCT GGA ATT GCA TGT TGC AAG GAG TCT 270
 271 CCT GGA TCT TCG CAC CcA GCT TCG GgT aCG GgA GGg AGT CcG GgT 315
 316 CCc GGC TAG GCc AGC ccg cag glg gag agg gtc ccc ggc agc ccc 360
 361 GCG CGC CCC TGG CCA TGT CTT TAA TGC CCT GCC CCT TCA TGT GGC 405
 406 CTT CTG AGG GTT CCC AGG GCT GGC CAG GGT TGT CTC CCA CCC GCG 450
 451 CGC GCC GTC TCA CCC CCA GCC AAA CCC ACC TGG CAG GGC TCC CTC 495
 496 CAG AAG AGA CCT TTT GAT TCC CGG CTC CCG CGC TCC CGC CTC CGC 540
 541 GCC AGC CCG GGA GGT GGC CCT GGA CAG CCG GAC CTC GCC CGG CCC 585
 586 CGG CTG NGG ACC ATG GTG TTT CTC TCG GGA AAT GCT TCC GAC AGC 630
 1 Met Val Phe Leu Ser Gly Asn Ala Ser Asp Ser 11
 631 TCC AAC TGC ACC CAA CCG CCG GCA CCG GTG AAC ATT TCC AAG GCC 675
 12 Ser Asn Cys Thr Gln Pro Pro Ala Pro Val Asn Ile Ser Lys Ala 26
 676 ATT CTG CTC GGG GTG ATC TTG GGG GGC CTC ATT CTT TTC GGG GTG 720
 27 Ile Leu Leu Gly Val Ile Leu Gly Gly Leu Ile Leu Phe Gly Val 41
 721 CTG GGT AAC ATC CTA GTG ATC CTC TCC GTA GCC TGT CAC CGA CAC 765
 42 Leu Gly Asn Ile Leu Val Ile Leu Ser Val Ala Cys His Arg His 56

FIG.21A

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766	CTG CAC TCA GTC ACG CAC TAC TAC ATC GTC AAC CTG GCG GTG GCC	810
57	Leu His Ser Val Thr His Tyr Tyr Ile Val Asn Leu Ala Val Ala	71
811	GAC CTC CTG CTC ACC TCC ACG GTG CTG CCC TTC TCC GCC ATC TTC	855
72	Asp Leu Leu Leu Thr Ser Thr Val Leu Pro Phe Ser Ala Ile Phe	86
856	GAG GTC CTA GGC TAC TGG GCC TTC GGC AGG GTC TTC TGC AAC ATC	900
87	Glu Val Leu Gly Tyr Trp Ala Phe Gly Arg Val Phe Cys Asn Ile	101
901	TGG GCG GCA GTG GAT GTG CTG TGC TGC ACC GCG TCC ATC ATG GGC	945
102	Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala Ser Ile Met Gly	116
946	CTC TGC ATC ATC TCC ATC GAC CGC TAC ATC GGC GTG AGC TAC CCG	990
117	Leu Cys Ile Ile Ser Ile Asp Arg Tyr Ile Gly Val Ser Tyr Pro	131
991	CTG CGC TAC CCA ACC ATC GTC ACC CAG AGG AGG GGT CTC ATG GCT	1035
132	Leu Arg Tyr Pro Thr Ile Val Thr Gln Arg Arg Gly Leu Met Ala	146
1036	CTG CTC TGC GTC TGG GCA CTC TCC CTG GTC ATA TCC ATT GGA CCC	1080
147	Leu Leu Cys Val Trp Ala Leu Ser Leu Val Ile Ser Ile Gly Pro	161
1081	CTC TTC GGC TGG AGG CAG CCG GCC CCC GAG GAC GAG ACC ATC TGC	1125
162	Leu Phe Gly Trp Arg Gln Pro Ala Pro Glu Asp Glu Thr Ile Cys	176
1126	CAG ATC AAC GAG GAG CCG GGC TAC GTG CTC TTC TCG GCT CTG GGC	1170
177	Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu Gly	191
1171	TCC TTC TAC CTG CCT CTG GCC ATC ATC CTG GTC ATG TAC TGC CGC	1215
192	Ser Phe Tyr Leu Pro Leu Ala Ile Ile Leu Val Met Tyr Cys Arg	206
1216	GTC TAC GTG GTG GCC AAG AGG GAG AGC CCG GGC CTC AAG TCT GGC	1260
207	Val Tyr Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly	221
1261	CTC AAG ACC GAC AAG TCG GAC TCG GAG CAA GTG ACG CTC CGC ATC	1305
222	Leu Lys Thr Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile	236
1306	CAT CGG AAA AAC GCC CCG GCA GGA GGC AGC GGG ATG GCC AGC GCC	1350
237	His Arg Lys Asn Ala Pro Ala Gly Gly Ser Gly Met Ala Ser Ala	251
1351	AAG ACC AAG ACG CAC TTC TCA GTG AGG CTC CTC AAG TTC TCC CGG	1395
252	Lys Thr Lys Thr His Phe Ser Val Arg Leu Leu Lys Phe Ser Arg	266

FIG.21B

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1396	GAG AAG AAA GCG GCC AAA ACG CTG GGC ATC GTG GTC GGC TGC TTC	1440
267	Glu Lys Lys Ala Ala Lys Thr Leu Gly Ile Val Val Gly Cys Phe	281
1441	GTC CTC TGC TGG CTG CCT TTT TTC TTA GTC ATG CCC ATT GGG TCT	1485
282	Val Leu Cys Trp Leu Pro Phe Phe Leu Val Met Pro Ile Gly Ser	296
1486	TTC TTC CCT GAT TTC AAG CCC TCT GAA ACA GTT TTT AAA ATA GTA	1530
297	Phe Phe Pro Asp Phe Lys Pro Ser Glu Thr Val Phe Lys Ile Val	311
1531	TTT TGG CTC GGA TAT CTA AAC AGC TGC ATC AAC CCC ATC ATA TAC	1575
312	Phe Trp Leu Gly Tyr Leu Asn Ser Cys Ile Asn Pro Ile Ile Tyr	326
1576	CCA TGC TCC AGC CAA GAG TTC AAA AAG GCC TTT CAG AAT GTC TTG	1620
327	Pro Cys Ser Ser Gln Glu Phe Lys Lys Ala Phe Gln Asn Val Leu	341
1621	AGA ATC CAG TGT CTC TGC AGA AAG CAG TCT TCC AAA CAT GCC CTG	1665
342	Arg Ile Gln Cys Leu CYS Arg Lys Gln Ser Ser Lys His Ala Leu	356
1666	GGC TAC ACC CTG CAC CCG CCC AGC CAG GCC GTG GAA GGG CAA CAC	1710
357	Gly Tyr Thr Leu His Pro Pro Ser Gln Ala Val Glu Gly Gln His	371
1711	AAG GAC ATG GTG CGC ATC CCC GTG GGA TCA AGA GAG ACC TTC TAC	1755
372	Lys Asp Met Val Arg Ile Pro Val Gly Ser Arg Glu Thr Phe Tyr	386
1756	AGG ATC TCC AAG ACG GAT GGC GTT TGT GAA TGG AAA TTT TTC TCT	1800
387	Arg Ile Ser Lys Thr Asp Gly Val Cys Glu Trp Lys Phe Phe Ser	401
1801	TCC ATG CCC CGT GGA TCT GCC AGG ATT ACA GTG TCC AAA GAC CAA	1845
402	Ser Met Pro Arg Gly Ser Ala Arg Ile Thr Val Ser Lys Asp Gln	416
1846	TCC TCC TGT ACC ACA GCC CGG GTG AGA AGT AAA AGC TTT TTG CAG	1890
417	Ser Ser Cys Thr Thr Ala Arg Val Arg Ser Lys Ser Phe Leu Gln	431
1891	GTC TGC TGC TGT GTA GGG CCC TCA ACC CCC AGC CTT GAC AAG AAC	1935
432	Val Cys Cys Cys Val Gly Pro Ser Thr Pro Ser Leu Asp Lys Asn	446
1936	CAT CAA GTT CCA ACC ATT AAG GTC CAC ACC ATC TCC CTC AGT GAG	1980
447	His Gln Val Pro Thr Ile Lys Val His Thr Ile Ser Leu Ser Glu	461
1981	AAC GGG GAG GAA GTC TAG	1998
462	Asn Gly Glu Glu Val End	466

FIG.21C

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CTCCCTGCCG GCCGCTCGTT CTGTGCCCCG GCCCGGCCAC CGACGGCCGG CGTTGAG ATGACT	6
TTC CGC GAT CTC CTG AGC GTC AGT TTC GAG GGA CCC CGC CCG GAC AGC AGCGCA	60
GGG GGC TCC AGC GCG GGC GGC GGC GGG GGC GGC GCG GGC GGC GCG GCC CCCTCG	114
GAG GGC CCG GCG GTG GGC GGC GTG CCG GGG GGC GCG GGC GGC GGC GGC GGC GGC GTG	168
GTG GGC GCA GGC AGC GGC GAG GAC AAC CGG AGC TCC GCG GGG GAG CCG GGGAGC	222
GCG GGC GCG GGC GGC GAC GTG AAT GGC ACG GCG GCC GTC GGG GGA CTG GTGGTG	276
AGC GCG CAG GGC GTG GGC GTG GGC GTC TTC CTG GCA GCC TTC ATC CTT ATGGCC	330
GTG GCA GGT AAC CTG CTT GTC ATC CTC TCA GTG GCC TGC AAC CGC CAC CTGCAG	384
ACC GTC ACC AAC TAT TTC ATC GTG AAC CTG GCC GTG GCC GAC CTG CTG CTGAGC	438
GCC ACC GTA CTG CCC TTC TCG GCC ACC ATG GAG GTT CTG GGC TTC TGG GCCTTT	492
GGC CGC GCC TTC TGC GAC GTA TGG GCC GCC GTG GAC GTG CTG TGC TGC ACGGCC	546
TCC ATC CTC AGC CTC TGC ACC ATC TCC GTG GAC CGG TAC GTG GGC GTG CGCCAC	600
TCA CTC AAG TAC CCA GCC ATC ATG ACC GAG CGC AAG GCG GCC GCC ATC CTGGCC	654
CTG CTC TGG GTC GTA GCC CTG GTG GTG TCC GTA GGG CCC CTG CTG GGC TGAAG	708
GAG CCC GTG CCC CCT GAC GAG CGC TTC TGC GGT ATC ACC GAG GAG GCG GGCTAC	762
GCT GTC TTC TCC TCC GTG TGC TCC TTC TAC CTG CCC ATG GCG GTC ATC GTGGTC	816
ATG TAC TGC CGC GTG TAC GTG GTC GCG CGC AGC ACC ACG CGC AGC CTC GAGGCG	870
GGC GTC AAG CGC GAG CGA GGC AAG GCC TCC GAG GTG GTG CTG CGC ATC CACTGT	924

FIG.22A

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CGC GGC GCG GCC ACG GGC GCC GAC GGG GCG CAC GGC ATG CGC AGC GCC AAGGGC 978
CAC ACC TTC CGC AGC TCG CTC TCC GTG CGC CTG CTC AAG TTC TCC CGT GAGAAG 1032
AAA GCG GCC AAG ACT CTG GCC ATC GTC GTG GGT GTC TTC GTG CTC TGC TGGTTC 1086
CCT TTC TTC TTT GTC CTG CCG CTC GGC TCC TTG TTC CCG CAG CTG AAG CCATCG 1140
GAG GGC GTC TTC AAG GTC ATC TTC TGG CTC GGC TAC TTC AAC AGC TGC GTGAAC 1194
CCG CTC ATC TAC CCC TGT TCC AGC CGC GAG TTC AAG CGC GCC TTC CTC CGTCTC 1248
CTG CGC TGC CAG TGC CGT CGT CGC CGG CGC CGC CGC CCT CTC TGG CGT GTCTAC 1302
GGC CAC CAC TGG CGG GCC TCC ACC AGC GGC CTG CGC CAG GAC TGC GCC CCGAGT 1356
TCG GGC GAC GCG CCC CCC GGA GCG CCG CTG GCC CTC ACC GCG CTC CCC GACCCC 1410
GAC CCC GAA CCC CCA GGC ACG CCC GAG ATG CAG GCT CCG GTC GCC AGC CGTCGA 1464
AAG CCA CCC AGC GCC TTC CGC GAG TGG AGG CTG CTG GGG CCG TTC CGG AGACCC 1518
ACG ACC CAG CTG CGC GCC AAA GTC TCC AGC CTG TCG CAC AAG ATC CGC GCCGGG 1572
GGC GCG CAG CGC GCA GAG GCA GCG TGC GCC CAG CGC TCA GAG GTG GAG GCTGTG 1626
TCC CTA GGC GTC CCA CAC GAG GTG GCC GAG GGC GCC ACC TGC CAG GCC TACGAA 1680
TTG GCC GAC TAC AGC AAC CTA CCG GAG ACC GAT ATT TAA 1719

FIG.22B

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F	R	D	L	L	S	V	S	F	E	G	P	R	P	D	S	M	T
G	G	S	S	A	G	G	G	G	G	G	A	G	G	A	G	S	A
E	G	P	A	V	G	G	G	P	G	G	A	G	G	G	P	S	S
V	G	A	A	S	G	E	G	N	R	S	S	A	G	E	P	P	V
A	G	A	Q	A	G	V	D	G	T	A	A	V	G	G	L	G	S
S	A	A	G	A	N	V	N	V	F	L	A	C	F	I	L	L	A
V	A	T	T	V	N	I	A	I	S	V	V	A	N	R	L	L	S
T	A	A	A	A	L	S	W	A	M	V	D	L	G	L	W	A	F
A	R	A	F	A	P	T	I	A	A	C	R	V	L	C	T	R	A
G	I	L	S	L	C	I	M	S	V	K	G	A	V	V	I	L	H
S	L	L	Y	L	A	L	V	T	S	I	I	P	A	R	G	W	A
S	L	P	V	P	D	C	R	V	F	V	P	T	L	E	V	G	Y
L	P	V	P	S	V	V	S	A	C	S	T	M	A	V	I	V	V
E	Y	C	R	R	R	G	K	A	R	D	V	T	R	S	L	E	A
A	V	K	A	A	G	A	S	G	S	I	G	V	L	S	I	A	C
M	G	A	R	F	V	L	V	V	A	L	L	M	F	S	R	C	K
G	T	F	K	F	S	P	I	V	R	V	V	K	V	L	W	P	F
R	A	F	F	F	L	I	L	G	S	L	F	P	Q	L	P	S	N
P	E	V	Y	V	V	S	F	W	A	G	Y	R	N	S	F	V	L
M	G	R	C	K	S	R	R	R	S	V	K	P	A	F	R	R	S
T	A	H	T	S	T	A	S	S	R	I	L	F	V	C	P	V	N
S	F	A	F	A	D	C	I	A	V	S	T	M	L	L	G	R	L
A	K	Y	V	V	R	G	L	V	G	L	V	V	F	S	L	S	L
K	Y	V	A	C	G	K	F	S	N	L	Y	S	P	R	P	G	V
V	A	C	G	K	F	S	N	L	Y	S	P	R	P	G	V	E	

FIG.23

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CTCCCTGCCG GCCGCTCGTT CTGTGCCCCG GCCCGGCCAC CGACGGCCGG CGTTGAG ATG ACT

6

M

T

TTC CGC GAT CTC CTG AGC GTC AGT TTC GAG GGA CCC CGC CCG GAC AGC AGC GCA 60
F R D L L S V S F E G P R P D S S A

GGG GGC TCC AGC GCG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC CCC TCG 114
G G S S A G G G G G G G A G G A A P S

GAG GGC CCG GCG GTG GGC GGC GTG CCG GGC GGC GGC GGC GGC GGC GGC GTG 168
E G P A V G G V P G G A G G G G G G V

GTG GGC GCA GGC AGC GGC GAG GAC AAC CCG AGC TCC GCG GGC GAG CCG GGC AGC 222
V G A G S G E D N R S S A G E P G S

*

GCG GGC GCG GGC GGC GAC GTG AAT GGC ACG GCG GCC GTC GGC GGA CTG GTG GTG 276
A G A G G D V N G T A A V G G L V V

*

AGC GCG CAG GGC GTG GGC GTG GGC GTC TTC CTG GCA GCC TTC ATC CTT ATG GCC 330
S A Q G V G V G V F L A A F I L M A

GTG GCA GGT AAC CTG CTT GTC ATC CTC TCA GTG GCC TGC AAC CGC CAC CTG CAG 384
V A G N L L V I L S V A C N R H L Q

FIG.24A

ACC GTC ACC AAC TAT TTC ATC GTG AAC CTG GCC GTG GCC GAC CTG CTG CTG AGC 438
T V T N Y F I V N L A V A D L L L S

GCC ACC GTA CTG CCC TTC TCG GCC ACC ATG GAG GTT CTG GGC TTC TGG GCC TTT 492
A T V L P F S A T M E V L G F W A F

GCC CGC GCC TTC TGC GAC GTA TGG GCC GCC GTG GAC GTG CTG TGC TGC ACG GCC 546
G R A F C D V W A A V D V L C C T A

TCC ATC CTC AGC CTC TGC ACC ATC TCC GTG GAC CGG TAC GTG GGC GTG CGC CAC 600
S I L S L C T I S V D R Y V G V R H

TCA CTC AAG TAC CCA GCC ATC ATG ACC GAG CGC AAG GCG GCC GCC ATC CTG GCC 654
S L K Y P A I M T E R K A A A I L A

CTG CTC TGG GTC GTA GCC CTG GTG GTG TCC GTA GGG CCC CTG CTG GGC TGG AAG 708
L L W V V A L V V S V G P L L G W K

GAG CCC GTG CCC CCT GAC GAG CGC TTC TGC GGT ATC ACC GAG GAG GCG GGC TAC 762
E P V P P D E R F C G I T E E A G Y

GCT GTC TTC TCC TCC GTG TGC TCC TTC TAC CTG CCC ATG GCG GTC ATC GTG GTC 816
A V F S S V C S F Y L P M A V I V V

ATG TAC TGC CGC GTG TAC GTG GTC GCG CGC AGC ACC ACG CGC AGC CTC GAG GCG 870
M Y C R V Y V V A R S T T R S L E A

FIG.24B

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GGC	GTC	AAG	CGC	GAG	CGA	GGC	AAG	GCC	TCC	GAG	GTG	GTG	CTG	CGC	ATC	CAC	TGT	924
G	V	K	R	E	R	G	K	A	S	E	V	V	L	R	I	H	C	

CGC	GGC	GCG	GCC	ACG	GGC	GCC	GAC	GGG	GCG	CAC	GGC	ATG	CGC	AGC	GCC	AAG	GGC	978
R	G	A	A	T	G	A	D	G	A	H	G	M	R	S	A	K	G	

CAC	ACC	TTC	CGC	AGC	TCG	CTC	TCC	GTG	CGC	CTG	CTC	AAG	TTC	TCC	CGT	GAG	AAG	1032
H	T	F	R	S	S	L	S	V	R	L	L	K	F	S	R	E	K	

AAA	GCG	GCC	AAG	ACT	CTG	GCC	ATC	GTG	GTG	GGT	GTG	TTC	GTG	CTC	TGC	TGG	TTC	1086
K	A	A	K	T	L	A	I	V	V	G	V	F	V	L	C	W	F	

CCT	TTC	TTC	TTT	GTG	CTG	CCG	CTC	GGC	TCC	TTG	TTC	CCG	CAG	CTG	AAG	CCA	TCG	1140
P	F	F	F	V	L	P	L	G	S	L	F	P	Q	L	K	P	S	

GAG	GGC	GTC	TTC	AAG	GTG	ATC	TTC	TGG	CTC	GGC	TAC	TTC	AAC	AGC	TGC	GTG	AAC	1194
E	G	V	F	K	V	I	F	W	L	G	Y	F	N	S	C	V	N	

CCG	CTC	ATC	TAC	CCC	TGT	TCC	AGC	CGC	GAG	TTC	AAG	CGC	GCC	TTC	CTC	CGT	CTC	1248
P	L	I	Y	P	C	S	S	R	E	F	K	R	A	F	L	R	L	

CTG	CGC	TGC	CAG	TGC	CGT	CGT	CGC	CGG	CGC	CGC	CGC	CCT	CTC	TGG	CGT	GTG	TAC	1302
L	R	C	Q	C	R	R	R	R	R	R	R	P	L	W	R	V	Y	

FIG.24C

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GGC CAC CAC TGG CGG GCC TCC ACC AGC GGC CTG CGC CAG GAC TGC GCC CCG AGT 1356
 G H H W R A S T S G L R Q D C A P S

TGG GGC GAC GCG CCC CCC GGA GCG CCG CTG GCC CTC ACC GCG CTC CCC GAC CCC 1410
 S G D A P P G A P L A L T A L P D P

GAC CCC GAA CCC CCA GGC ACG CCC GAG ATG CAG GCT CCG GTC GCC AGC CGT CGA 1464
 D P E P P G T P E M Q A P V A S R R

AAG CCA CCC AGC GCC TTC CCG GAG TGG AGG CTG CTG GGG CCG TTC CCG AGA CCC 1518
 K P P S A F R E W R L L G P F R R P

ACG ACC CAG CTG CGC GCC AAA GTC TCC AGC CTG TCG CAC AAG ATC CCG GCC GGG 1572
 T T Q L R A K V S S L S H K I R A G

GGC GCG CAG CGC GCA GAG GCA GCG TGC GCC CAG CGC TCA GAG GTG GAG GCT GTG 1626
 G A Q R A E A A C A Q R S E V E A V

TCC CTA GGC GTC CCA CAC GAG GTG GCC GAG GGC GCC ACC TGC CAG GCC TAC GAA 1680
 S L G V P H E V A E G A T C Q A Y E

TTG GCC GAC TAC AGC AAC CTA CGG GAG ACC GAT ATT TAA 1719
 L A D Y S N L R E T D I .

FIG.24D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/04590

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/170, 257, 278, 302, 321, 322, 323, 193; 544/283; 546/17, 200, 197, 198, 199, 115, 271; 546/198, 199; 514/321, 322

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BEILSTEIN, CHEMICAL ABSTRACTS (CA)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR, A, 2,621,588 (BOAVENTURA ET AL.) 18 OCTOBER 1987, see entire document.	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A document defining the general state of the art which is not considered to be of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G	document member of the same patent family
*O document referring to an oral disclosure, use, exhibition or other means		
*P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

20 JULY 1995

Date of mailing of the international search report

08 AUG 1995

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04590**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 12, 16, 17, 20, and 23-26 (in-part)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04590A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07D 401/04, 413/04, 471/04, 471/10; A61K 31/415, 31/42, 31/445, 31/44, 31/47, 31/505

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/170, 257, 278, 302, 321, 322, 323, 193; 544/283; 546/17, 200, 197, 198, 199, 115, 271

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Invention I: Claims 1-7, 12, 16, 17, 20, and 23-26 in-part drawn to compounds, pharmaceutical compositions and methods of treating benign prostatic hyperplasia wherein the term (X) in the depicted formula(a) represents [4-(2-oxo-3-benzoxazoliny)], 4-(2-oxo-3-benzthiazoliny), 4-(1,3-dihydro-2-oxo-benzimidazolin-1-yl) all optionally substituted as defined; the term (E) in the main formula represents carbonyl or sulfonyl, the term (Y) in the main formula represents sulfonyl; the terms (A, B, G, D) in the main formula represent carbon; the terms R1, R2, R3, and R4 and the integer (n) in the main formula are as defined, the term (Q) in main formula represents (CH₂)_r wherein r is one. This invention is classified in Class 546, subclasses 198 and 199; class 514, subclasses 321 and 322.

Invention II: Claims 1-7, 12, 16, 17, 20, and 23-26 (in-part) drawn to compounds, pharmaceutical compositions, and methods of treating benign prostatic hyperplasia except that the term (X) represents optionally substituted 4-(2-oxo-(1H)quinolin-1-yl) 4-(3,4-dihydro-2-oxo-(1H)quinolin-1-yl) and 4-(1,3-dihydro-2-oxo-(1H)-3,4-dihydro-quinazolin-1-yl) moieties classified in Class 544; subclass 283; Class 546, subclass 200; Class 514, subclasses 257 and 312, respectively.

Invention III: Claims 1-7, 12, 16, 17, 20, and 23-26 (in-part) drawn to compounds, pharmaceutical compositions, and methods of treating benign prostatic hyperplasia except that the term (X) represents 3, 3a, 8, 8a-tetrahydro-2H-indeno [1,2-d]-oxazoliny or 4-(2-oxo-naphth-[2,3-d]oxazoliny) moieties. This invention is classified in Class 198; Class 514, subclass 321.

Invention IV: Claims 1-7, 12, 16, 17, 20, and 23-26 (in-part), drawn to compounds, pharmaceutical compositions, and methods of treating benign prostatic hyperplasia and Invention I, except that the term (X) represents 4-(2-oxo-3-oxazolo[4,5-b]pyridine). This invention is classified in Class 546, subclass 115; Class 514, subclass 302.

Invention V: Claims 1-7, 12, 16, 17, 20, and 23-26 (in-part) drawn to compounds, pharmaceutical compositions, and methods of treating benign prostatic hyperplasia and except that spiro compounds are involved according to formula (b) of claim one. This invention is classified in Class 546, subclass 17; Class 514, subclass 278.

Invention VI: Claims 1, 2, 12, 16, 17, 20, and 23-26 (in-part) drawn to compounds, pharmaceutical compositions, and methods of treating benign prostatic hyperplasia except that the 1,2-benzisothiazol-3(2H)one moiety is replaced by a phthalimide ring. Class 546, subclass 271; Class 514, subclass 323.

Invention VII: Claims 1, 2, 12, 16, 17, 20, and 23-26 (in-part) except that it includes all of the residual compounds not previously defined. This invention is classified in plethora of subclasses in Classes 540, 544, 546, 548, and 549.

Invention VIII: Claims 8-11, 13-15, 18, 19, 21, and 22 (in-part) drawn to pharmaceutical compositions and methods of use comprising a compound according to Inventions I-IV and an additional testosterone 5-alpha reductase inhibitor classified in Class 514, subclasses 17-, 257, 302, 312, 321, and 322.

Invention IX: Claims 8-11, 13-15, 18, 19, 21, and 22 (in-part) drawn to pharmaceutical compositions and methods of use comprising a spiral compound according to one of Inventions V-VIII and an additional testosterone 5-alpha reductase inhibitor. This invention is classified in Class 514, subclasses 170, 193, 278, and 323.

Lack of Unity of Invention has been found to exist since each invention is structurally dissimilar, lacking common core structure as is evidenced by the separate classification, and thus, not so linked as to form a single inventive concept. Furthermore, the technical relationship between each invention is substantially different.